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FORM PTO-100  
(REV 10-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371**

4951US

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

**09/868732**

INTERNATIONAL APPLICATION NO.  
PCT/EP99/10209

INTERNATIONAL FILING DATE  
16 December 1999 (16.12.99)

PRIORITY DATE CLAIMED  
16 December 1998 (16.12.98)

TITLE OF INVENTION

**SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS**

APPLICANT(S) FOR DO/EO/US **Leif ANDERSSON, Michel GEORGES, Geert SPINCEMAILLE,  
Carine Danielle Andree NEZER**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
  2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
  3. ☐ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
  4. ☐ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
  5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
    - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
    - b. ☐ has been communicated by the International Bureau.
    - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
  6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
    - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
    - b. ☐ have been communicated by the International Bureau.
    - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
    - d. ☐ have not been made and will not be made.
  8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
  9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
  10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
- Items 11 to 16 below concern document(s) or information included:**
11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
  12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
  13. ☒ A **FIRST** preliminary amendment.  
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
  14. ☐ A substitute specification.
  15. ☐ A change of power of attorney and/or address letter.
  16. ☒ Other items or information:  
Copy of Preliminary Examination Report

**NOTICE OF EXPRESS MAILING**

Express Mail Mailing Label Number: EL740537159US

Date of Deposit with USPS: June 15, 2001

Person making Deposit: Jared Turner

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) <b>097868732</b>		INTERNATIONAL APPLICATION NO. <b>PCT/EP99/10209</b>		ATTORNEY'S DOCKET NUMBER <b>4951US</b>	
<b>17. <input checked="" type="checkbox"/> The following fees are submitted:</b> <b>BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... <b>\$1000.00</b> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO..... <b>\$860.00</b> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... <b>\$710.00</b> International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... <b>\$690.00</b> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... <b>\$100.00</b> <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>CALCULATIONS</b> PTO USE ONLY	
				\$ 860.00	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$ 130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	32 - 20 =	12	X \$18.00	\$ 226.00	
Independent claims	3 - 3 =	0	X \$80.00	\$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$ 1,216.00	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$	
<b>SUBTOTAL =</b>				\$ 1,216.00	
Processing fee of <b>\$130.00</b> for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
<b>TOTAL NATIONAL FEE =</b>				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). <b>\$40.00</b> per property				\$	
<b>TOTAL FEES ENCLOSED =</b>				\$ 1,216.00	
				Amount to be refunded:	\$
				charged:	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>1,216.00</u> to cover the above fees is enclosed.					
b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>20-1469</u> . A duplicate copy of this sheet is enclosed.					
<b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
SEND ALL CORRESPONDENCE TO: Allen C. Turner TRASKBRITT, P.C. P.O. Box 2550 Salt Lake City, Utah United States of America					
				SIGNATURE:	<u>FOR</u>
				NAME	Allen C. Turner
				REGISTRATION NUMBER	33,041

**PATENT**  
**4951US**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**In re Application of:**

Andersson et al.

**Serial No.:** To be assigned

**Filed:** 15 June 2001

**For:** SELECTING ANIMALS FOR  
PARENTALLY IMPRINTED TRAITS

**Examiner:** To be assigned

**Group Art Unit:** To be assigned

**Attorney Docket No.:** 4951US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: EL74037159US

Date of Deposit with USPS: 15 June 2001

Person making Deposit: Jared Turner

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Please amend the above identified patent application as follows:

**IN THE CLAIMS:**

Claims 18-23 have been cancelled and new claims 28-38 have been added. Claims 1-17 and 24-27 have been amended herein. All cancellations of and amendments to claims are made without prejudice or disclaimer. All of the pending claims 1-17 and 24-38 are presented, pursuant to 37 C.F.R. §§ 1.121(c)(1)(i) and 1.121(c)(3), in clean form below. Please enter these claims as amended. Also attached is a marked-up version of the claims amended herein pursuant to 37 C.F.R. § 1.121(c)(1)(ii).

1. (Amended) A method for selecting an animal for having desired genotypic properties comprising testing said animal for the presence of a parentally imprinted quantitative trait locus (QTL).

2. (Amended) The method according to claim 1, further comprising testing a nucleic acid sample from said animal for the presence of a QTL.

3. (Amended) The method according to claim 1 wherein said animal comprises a pig and in said pig said QTL is located at chromosome 2.

4. (Amended) The method according to claim 1 wherein said animal comprises a pig and in said pig said QTL maps at about position 2p1.7.

5. (Amended) The method according to claim 1 wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.

6. (Amended) The method according to claim 5 wherein said QTL comprises at least a part of an insulin-like growth factor-2 gene.

7. (Amended) The method according to claim 1 wherein said animal comprises a pig and in said pig said QTL comprises a marker characterized as nt241 (G-A) or as Swc9.

8. (Amended) The method according to claim 1 wherein a paternal allele of said QTL is predominantly expressed in said animal.

9. (Amended) The method according to claim 1 wherein a maternal allele of said QTL is predominantly expressed in said animal.



10. (Amended) An isolated and/or recombinant nucleic acid comprising a parentally imprinted quantitative trait locus (QTL) or a functional fragment of said QTL comprising genetic information capable of influencing a quantitative trait of an animal.

11. (Amended) An isolated and/or recombinant nucleic acid comprising a synthetic parentally imprinted quantitative trait locus (QTL) derived from at least one chromosome or a functional fragment of said chromosome comprising genetic information capable of influencing a quantitative trait of an animal.

12. (Amended) The isolated and/or recombinant nucleic acid according to claim 10 at least partly derived from a *Sus scrofa* chromosome.

13. (Amended) The isolated and/or recombinant nucleic acid according to claim 12 wherein said nucleic acid is at least partly derived from a *Sus scrofa* chromosome 2.

14. (Amended) The nucleic acid according to claim 10 wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.

15. (Amended) The nucleic acid according to claim 10 wherein said QTL comprises at least a part of an insulin-like growth factor-2 gene.

16. (Amended) The nucleic acid according to claim 10 wherein a paternal allele of said QTL is capable of being predominantly expressed.

17. (Amended) The nucleic acid according to claim 10 wherein a maternal allele of said QTL is capable of being predominantly expressed.

24. (Amended) A transgenic animal comprising the isolated and/or recombinant nucleic acid according to claim 11.

25. (Amended) The transgenic animal according to claim 24 wherein said transgenic animal is a male.

26. (Amended) Sperm or an embryo derived from the transgenic animal according to claim 24.

27. (Amended) A method for breeding animals destined for slaughter comprising utilizing the sperm or embryo according to claim 26.

28. (New) The isolated and/or recombinant nucleic acid according to claim 13 wherein said isolated and/or recombinant nucleic acid is at least partly derived from a region mapping at about position 2p1.7.

29. (New) The isolated and/or recombinant nucleic acid according to claim 11 at least partly derived from a *Sus scrofa* chromosome.

30. (New) The isolated and/or recombinant nucleic acid according to claim 29 wherein said isolated and/or recombinant nucleic acid is at least partly derived from a *Sus scrofa* chromosome 2.

31. (New) The isolated and/or recombinant nucleic acid according to claim 30 wherein said isolated and/or recombinant nucleic acid is at least partly derived from a region mapping at about position 2p1.7.

32. (New) The isolated and/or recombinant nucleic acid according to claim 11 wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.

33. (New) The isolated and/or recombinant nucleic acid according to claim 11 wherein said QTL comprises at least a part of an insulin-like growth factor-2 gene.

34. (New) The isolated and/or recombinant nucleic acid according to claim 11 wherein a paternal allele of said QTL is capable of being predominantly expressed.

35. (New) The isolated and/or recombinant nucleic acid according to claim 11 wherein a maternal allele of said QTL is capable of being predominantly expressed.

36. (New) The method according to claim 1 wherein said testing comprises utilizing an isolated and/or recombinant nucleic acid comprising a QTL or a functional fragment of said QTL comprising genetic information capable of influencing a quantitative trait of said animal.

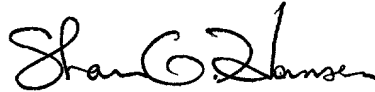
37. (New) The method according to claim 36 wherein said animal comprises a breeding animal or an animal destined for slaughter and wherein said desired genotypic properties further comprise potential phenotypic properties.

38. (New) The method according to claim 37 wherein said desired genotypic properties are related to muscle mass and/or fat deposition.

## REMARKS

The application is to be amended without prejudice or disclaimer as previously set forth, which should not be viewed as narrowing or limiting the claims. The amendments are sought to conform the application to a form more consistent with Office practice by removing multiple dependencies. It is respectfully submitted that no new matter has been added by the amendments. Should the Office determine that additional issues remain, which might be resolved by a telephone conference, it is respectfully invited to contact applicants' undersigned attorney.

Respectfully Submitted,



Shawn G. Hansen  
Registration Number 42,627  
Attorney for Applicants  
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Date: 15 June 2001

Enclosure: Version With Markings to Show Changes Made

N:\2183\4951\Preliminary Amendment.wpd

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

1. (Amended) A method for selecting [a domestic] an animal for having desired genotypic properties comprising testing said animal for the presence of a parentally imprinted quantitative trait locus (QTL).

2. (Amended) [A] The method according to claim 1, further comprising testing a nucleic acid sample from said animal for the presence of a [parentally imprinted quantitative trait locus ([QTL])].

3. (Amended) [A] The method according to claim 1 [or 2 ]wherein said animal comprises a pig and in [the] said pig said QTL is located at chromosome 2.

4. (Amended) [A] The method according to claim [2 or 3] 1 wherein said animal comprises a pig and in said pig said QTL [is mapping] maps at [around] about position 2p1.7.

5. (Amended) [A] The method according to claim 1 [to 4] wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.

6. (Amended) [A] The method according to claim 5 wherein said QTL comprises at least a part of an insulin-like growth factor-2 [(IGF2)]gene.

7. (Amended) [A] The method according to [anyone of claims] claim 1 [to 6] wherein said animal comprises a pig and in [the] said pig said QTL comprises a marker characterized as nt241(G-A) or as Swc9[, as identified in figure 4].

8. (Amended) [A] The method according to [anyone of claims 1-7] claim 1 wherein a paternal allele of said QTL is predominantly expressed in said animal.

9. (Amended) [A] The method according to [anyone of claims 1-7] claim 1 wherein a maternal allele of said QTL is predominantly expressed in said animal.

10. (Amended) An isolated and/or recombinant nucleic acid comprising a parentally imprinted quantitative trait locus (QTL) or a functional fragment [derived thereof] of said QTL comprising genetic information capable of influencing a quantitative trait of an animal.

11. (Amended) An isolated and/or recombinant nucleic acid comprising a synthetic parentally imprinted quantitative trait locus (QTL) derived from at least one chromosome or a functional fragment [derived thereof.] of said chromosome comprising genetic information capable of influencing a quantitative trait of an animal.

12. (Amended) [A] The isolated and/or recombinant nucleic acid according to claim 10 [or 11] at least partly derived from a Sus scrofa chromosome.

13. (Amended) [A] The isolated and/or recombinant nucleic acid according to claim 12 wherein said nucleic acid is at least partly derived from a Sus scrofa chromosome 2[, preferably from a region mapping at around position 2p1.7].

14. (Amended) [A] The nucleic acid according to [any one of claims] claim 10 [to 13] wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.

15. (Amended) [A] The nucleic acid according to [any one of claims] claim 10 [to 14] wherein said QTL comprises at least a part of [a] an insulin-like growth factor-2 [(IGF2) ]gene.

16. (Amended) [A] The nucleic acid according to [anyone of claims] claim 10 [to 15] wherein a paternal allele of said QTL is capable of being predominantly expressed.

17. (Amended) [A] The nucleic acid according to [anyone of claims] claim 10 [to 16] wherein a maternal allele of said QTL is capable of being predominantly expressed.

24. (Amended) A transgenic animal comprising the isolated and/or recombinant [a] nucleic acid according to claim 11 [anyone of claims 11 to 16].

25. (Amended) [An animal]The transgenic animal according to [anyone of claims 21-24 which]claim 24 wherein said transgenic animal is a male.

26. (Amended) Sperm or an embryo derived from [an]the transgenic animal according to [anyone of claims 21-25]claim 24.

27. (Amended) [Use of a sperm or an embryo according to claim 26 in] A method for breeding animals destined for slaughter comprising utilizing the sperm or embryo according to claim 26.



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**In re Application of:**

ANDERSSON et al.

**Serial No.:** 09/868,732

**Filed:** June 15, 2001

**For:** SELECTING ANIMALS FOR  
PARENTALLY IMPRINTED TRAITS

**Examiner:** To be assigned

**Group Art Unit:** To be assigned

**Attorney Docket No.:** 4951US

CERTIFICATE OF MAILING

I hereby certify that this correspondence along with any attachments referred to or identified as being attached or enclosed is being deposited with the United States Postal Service as First Class Mail (under 37 C.F.R. § 1.8(a)) on the date of deposit shown below with sufficient postage and in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231

9/17/01  
Date of Deposit

Signature of registered practitioner or other person  
having reasonable basis to expect mailing to occur on  
date of deposit shown pursuant to 37 C.F.R. §  
1.8(a)(1)(ii)

Daniel Thatcher  
Typed/printed name of person whose signature is  
contained above

AMENDMENT

Box Non-Fee Amendment  
Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced patent application on the merits, entry of the amendments as set forth herein is respectfully solicited.



IN THE SPECIFICATION:

Pursuant to 37 C.F.R. §§ 1.121 and 1.125 (as amended to date) please enter the substitute specification in clean form and including paragraph numbers [0001] through [0085] attached hereto as Appendix A. A marked-up substitute specification to clearly identify amendments to the specification as required by 37 C.F.R. § 1.121(b)(3)(iii) is attached as Appendix B. It is respectfully submitted that the substitute specification does not introduce new matter into the above-referenced patent application.

**REMARKS**

No new matter has been added. The Applicants again request entry of the amendments as set forth in the Appendices hereto prior to examination of the application on the merits.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Allen C. Turner', with a long horizontal flourish extending to the right.

Allen C. Turner

Registration No. 33,041

Attorney for Applicants

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P. O. Box 2550

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Telephone: (801) 532-1922

Date: September 17, 2001

ACT/dn

N:\2183\4951\Amendment - 091501

# **APPENDIX A**

**(CLEAN VERSION OF SUBSTITUTE SPECIFICATION EXCLUDING CLAIMS)**

**(Serial No. 09/868,732)**

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: \_\_\_\_\_

Date of Deposit with USPS: \_\_\_\_\_

Person making Deposit: \_\_\_\_\_

APPLICATION FOR LETTERS PATENT

for

**SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS**

Inventor:

Andersson, Leif  
Georges, Michel  
Spincemaille, Geert

Attorney:  
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Registration No. 33,041  
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Salt Lake City, Utah 84110  
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## SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS

### TECHNICAL FIELD

**[0001]** The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition.

### BACKGROUND OF THE INVENTION

**[0002]** Breeding schemes for domestic animals have so far focused on farm performance traits and carcass quality. This has resulted in substantial improvements in traits like reproductive success, milk production, lean/fat ratio, prolificacy, growth rate and feed efficiency. Relatively simple performance test data have been the basis for these improvements, and selected traits were assumed to be influenced by a large number of genes, each of small effect (the infinitesimal gene model). There are now some important changes occurring in this area. First, the breeding goal of some breeding organisations has begun to include meat quality attributes in addition to the “traditional” production traits. Second, evidence is accumulating that current and new breeding goal traits may involve relatively large effects (known as major genes), as opposed to the infinitesimal model that has been relied on so far.

**[0003]** Modern DNA-technologies provide the opportunity to exploit these major genes, and this approach is a very promising route for the improvement of meat quality, especially since direct meat quality assessment is not viable for potential breeding animals. Also for other traits such as lean/fat ratio, growth rate and feed efficiency, modern DNA technology can be very effective. Also these traits are not always easy to measure in the living animal.

**[0004]** The evidence for several of the major genes was originally obtained using segregation analysis, i.e., without any DNA marker information. Afterwards, molecular studies were performed to detect the location of these genes on the genetic map. In practice, and except for alleles of very large effect, DNA studies are required to dissect the genetic nature of most traits of economic importance. DNA markers can be used to localise genes or alleles responsible for qualitative traits like coat color, and they can also be used to detect genes or alleles with substantial effects on

quantitative traits like growth rate, IMF, etc. In this case, the approach is referred to as QTL (quantitative trait locus) mapping, wherein a QTL comprises at least a part of the nucleic acid genome of an animal where genetic information capable of influencing said quantitative trait (in said animal or in its offspring) is located. Information at the DNA level can not only help to fix a specific major gene in a population, but also assist in the selection of a quantitative trait which is already selected for. Molecular information in addition to phenotypic data can increase the accuracy of selection and therefore the selection response.

**[0005]** Improving meat quality or carcass quality is not just about changing levels of traits like tenderness or marbling, but it is also about increasing uniformity. The existence of major genes provides excellent opportunities for improving meat quality because it allows large steps to be made in the desired direction. It will help to reduce variation, since we can fix relevant genes in our products. Another aspect is that selecting for major genes allows differentiation for specific markets. Studies are underway in several species, particularly, pigs, sheep, deer and beef cattle.

**[0006]** In particular, intense selection for meat production has resulted in animals with extreme muscularity and leanness in several livestock species. In recent years it has become feasible to map and clone several of the genes causing these phenotypes, paving the way towards more efficient marker-assisted selection, targeted drug development (performance-enhancing products) and transgenesis. Mutations in the ryanodine receptor (Fuji et al, 1991; MacLennan and Phillips, 1993) and myostatin (Grobet et al, 1997; Kambadur et al, 1997; McPherron and Lee, 1997) have been shown to cause muscular hypertrophies in pigs and cattle respectively, while genes with major effects on muscularity and/or fat deposition have, for instance, been mapped to pig chromosome 4 (Andersson et al, 1994) and sheep chromosome 18 (Cocket et al, 1996).

## DISCLOSURE OF THE INVENTION

**[0007]** However, although there have been successes in identifying QTLs, the information is currently of limited use within commercial breeding programmes. Many workers in this field conclude that it is necessary to identify the particular genes underlying the QTL. This is a substantial task, as the QTL region is usually relatively large and may contain many genes. Identification of the relevant genes from the many that may be involved thus remains a significant hurdle in farm animals.

[0008] The invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising testing the animal for the presence of a parentally imprinted qualitative or quantitative trait locus (QTL). Herein, a domestic animal is defined as an animal being selected or having been derived from an animal having been selected for having desired genotypic or potential phenotypic properties.

[0009] Domestic animals provide a rich resource of genetic and phenotypic variation; traditionally, domestication involves selecting an animal or its offspring for having desired genotypic or potential phenotypic properties. This selection process has in the past century been facilitated by growing understanding and utilisation of the laws of Mendelian inheritance. One of the major problems in breeding programmes of domestic animals is the negative genetic correlation between reproductive capacity and production traits. This is, for example, the case in cattle (a high milk production generally results in slim cows and bulls), poultry (broiler lines have a low level of egg production and layers have generally very low muscle growth), pigs (very prolific sows are, in general, fat and have comparatively less meat) or sheep (high prolific breeds have low carcass quality and vice versa). The invention now provides that knowledge of the parental imprinting character of various traits allows to select, for example, sire lines homozygous for a paternally imprinted QTL, for example, linked with muscle production or growth; the selection for such traits can thus be less stringent in dam lines in favour of the reproductive quality. The phenomenon of genetic or parental imprinting has never been utilised in selecting domestic animals; it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. The invention provides a breeding programme, wherein knowledge of the parental imprinting character of a desired trait, as demonstrated herein, results in a breeding programme, for example, a BLUP programme, with a modified animal model. This increases the accuracy of the breeding value estimation and speeds up selection compared to conventional breeding programmes. Until now, the effect of a parentally imprinted trait in the estimation of a conventional BLUP programme was neglected; using and understanding the parental character of the desired trait, as provided by the invention, allows selecting on parental imprinting, even without DNA testing. For example, selecting genes characterised by paternal imprinting is provided to help increase uniformity; a (terminal) parent homozygous for the “good or wanted” alleles will pass them to all offspring, regardless of the other parent’s alleles, and

the offspring will all express the desired parent's alleles. This results in more uniform offspring. Alleles that are interesting or favourable from the maternal side are often the ones that have opposite effects to alleles from the paternal side. For example, in meat animals such as pigs, alleles linked with meat quality traits such as intramuscular fat or muscle mass could be fixed in the dam lines while alleles linked with reduced backfat could be fixed in the sire lines. Other desirable combinations are, for example, fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

**[0010]** In a preferred embodiment, the invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising testing a nucleic acid sample from the animal for the presence of a parentally imprinted quantitative trait locus (QTL). A nucleic acid sample can, in general, be obtained from various parts of the animal's body by methods known in the art. Traditional samples for the purpose of nucleic acid testing are blood samples or skin or mucosal surface samples, but samples from other tissues can be used as well; in particular, sperm samples, oocyte or embryo samples can be used. In such a sample, the presence and/or sequence of a specific nucleic acid, be it DNA or RNA, can be determined with methods known in the art, such as hybridisation or nucleic acid amplification or sequencing techniques known in the art. The invention provides testing such a sample for the presence of nucleic acid wherein a QTL or allele associated therewith is associated with the phenomenon of parental imprinting, for example, where it is determined whether a paternal or maternal allele of said QTL is capable of being predominantly expressed in the animal.

**[0011]** The purpose of breeding programmes in livestock is to enhance the performances of animals by improving their genetic composition. In essence, this improvement accrues by increasing the frequency of the most favourable alleles for the genes influencing the performance characteristics of interest. These genes are referred to as QTL. Until the beginning of the nineties, genetic improvement was achieved via the use of biometrical methods, but without molecular knowledge of the underlying QTL.

**[0012]** Since the beginning of the nineties and due to recent developments in genomics, it is conceivable to identify the QTL underlying a trait of interest. The invention now provides identifying and using parentally imprinted QTLs which are useful for selecting animals by mapping quantitative



trait loci. Again, the phenomenon of genetic or paternal imprinting has never been utilised in selecting domestic animals; it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. For example, Kovacs and Kloting (Biochem. Mol. Biol. Int. 44:399-405, 1998), where parental imprinting is not mentioned, and not suggested, found linkage of a trait in female rats, but not in males, suggesting a possible sex specificity associated with a chromosomal region, which, of course, excludes parental imprinting, a phenomenon wherein the imprinted trait of one parent is preferably but genderaspecifically expressed in his or her offspring.

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

### Legends to the Figures

**[0013]** Fig. 1: Test statistic curves obtained in QTL analyses of chromosome 2 in a Wild Boar/Large White intercross. The graph plots the F ratio testing the hypothesis of a single QTL at a given position along the chromosome for the traits indicated. The marker map with the distances between markers in Kosambi centiMorgan is given on the X-axis. The horizontal lines represent genome-wise significant ( $P < 0.05$ ) and suggestive levels for the trait lean meat in ham; similar significance thresholds were obtained for the other traits.

**[0014]** Figure 2: Piétrain pig with characteristic muscular hypertrophy.

**[0015]** Figures 3A-3C: Lodscore curves obtained in a Piétrain x Large White intercross for six phenotypes measuring muscle mass and fat deposition on pig chromosome 2. The most likely positions of the *IGF2* and *MyoD* genes determined by linkage analysis with respect to the microsatellite marker map are shown.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL. 3A:  $\log_{10}(H_1/H_0)$ , 3B:  $\log_{10}(H_2/H_0)$ , 3C:  $\log_{10}(H_3/H_0)$ .

**[0016]** Figure 4: A. Structure of the human *IGF2* gene according to ref. 17, with aligned porcine adult liver cDNA sequence as reported in ref. 16. The position of the *nt241(G-A)* transition and *Swc9* microsatellite are shown. B. The corresponding markers were used to demonstrate the

monoallelic (paternal) expression of IGF2 in skeletal muscle and liver of 10-week old fetuses. PCR amplification of the *nt421* (G-A) polymorphism and *Swc9* microsatellite from genomic DNA clearly shows the heterozygosity of the fetus, while only the paternal allele is detected in liver cDNA (*nt421*(G-A) and *Swc9*) and muscle cDNA (*Swc9*). The absence of RT-PCR product for *nt421*(G-A) in fetal muscle points towards the absence of mRNA including exon 2 in this tissue. Parental origin of the fetal alleles was determined from the genotypes of the sire and dam (data not shown).

[0017] Figure 5: A NotI restriction map showing the relative position of BAC-PIGF2-1 (comprising INS and IGF2 genes), and BAC-PIGF2-2 (comprising IGF2 and H19 genes).

[0018] Figure 6: Nucleic acid sequences of contig 1 to contig 115 derived from BAC-PIGF2-1 which was shotgun sequenced using standard procedures and automatic sequencers.

[0019] Figure 7: Similarity between porcine contigs of figure 6 and orthologous sequences in human.

[0020] Figure 8: Nucleic acid sequences of contig 1 to contig 7 derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1) which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

[0021] Figure 9: Similarity between porcine contigs of figure 8 and orthologous sequences in human.

[0022] Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals.

## DETAILED DESCRIPTION OF THE INVENTION

[0023] The invention provides the initial localisation of a parentally imprinted QTL on the genome by linkage analysis with genetic markers, and the actual identification of the parentally imprinted gene(s) and causal mutations therein. Molecular knowledge of such a parentally imprinted QTL allows for more efficient breeding designs herewith provided. Applications of molecular knowledge of parentally imprinted QTLs in breeding programmes include: marker-assisted segregation analysis to identify the segregation of functionally distinct, parentally imprinted QTL alleles in the populations of interest, marker-assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy or selection intensity or by reducing the

generation interval using the understanding of the phenomenon of parental imprinting, marker-assisted introgression (MAI) to efficiently transfer favourable parentally imprinted QTL alleles from a donor to a recipient population, genetic engineering of the identified parentally imprinted QTL and genetic modification of the breeding stock using transgenic technology, and development of performance enhancing products using targeted drug development exploiting molecular knowledge of said QTL.

**[0024]** The inventors undertook two independent experiments to determine the practical use of parental imprinting of a QTL.

**[0025]** In a first experiment, performed in a previously described Piétrain x Large White intercross, the likelihood of the data were computed under a model of paternal (paternal allele only expressed) and maternal imprinting (maternal allele only expressed) and compared with the likelihood of the data under a model of a conventional "Mendelian" QTL. The results strikingly demonstrated that the QTL was indeed paternally expressed, the QTL allele (Piétrain or Large White) inherited from the F<sub>1</sub> sow having no effect whatsoever on the carcass quality and quantity of the F<sub>2</sub> offspring. It was seen that very significant lodscores were obtained when testing for the presence of a paternally expressed QTL, while there was no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. The same tendency was observed for all traits, showing that the same imprinted gene is responsible for the effects observed on the different traits. Table 1 reports the maximum likelihood (ML) phenotypic means for the F<sub>2</sub> offspring sorted by inherited paternal QTL allele.

**[0026]** In a second experiment performed in the Wild Boar X Large White intercross, QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the chromosome 2 map using a statistical model testing for the presence of an imprinting effect. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; Table I). The clear paternal expression of a QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). For a given paternally imprinted QTL, implementation of marker-assisted segregation analysis, selection (MAS) and introgression (MAI) can be performed using genetic markers that are linked to the QTL,

using genetic markers that are in linkage disequilibrium with the QTL, or using the actual causal mutations within the QTL.

[0027] Understanding the parent-of-origin effect characterising a QTL allows for its optimal use in breeding programmes. Indeed, marker-assisted segregation analysis under a model of parental imprinting will yield better estimates of QTL allele effects. Moreover, it allows for the application of specific breeding schemes to optimally exploit a QTL. In one embodiment of the invention, the most favourable QTL alleles would be fixed in breeding animal lines and, for example, used to generate commercial, crossbred males by marker-assisted selection (MAS, within lines) and marker-assisted introgression (MAI, between lines). In another embodiment, the worst QTL alleles would be fixed in the animal lines used to generate commercial crossbred females by MAS (within lines) and MAI (between lines).

[0028] In a preferred embodiment of the invention, the animal is a pig. Note, for example, that the invention provides the insight that today half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit an unfavourable Large White muscle mass QTL as provided by the invention, causing considerable loss, and the invention now, for example, provides the possibility to select the better half of the population in that respect. However, it is also possible to select commercial sow lines enriched within the boars unfavourable alleles, allowing the sows to be equipped with other alleles more desirable for, for example, reproductive purposes.

[0029] In a preferred embodiment of a method provided by the invention, said QTL is located at a position corresponding to a QTL located at chromosome 2 in the pig. For example, it is known to form comparative mapping data between pig and human, including bidirectional chromosome painting and that SSC2p is homologous to HSA11pter-q13<sup>11, 12</sup>. HSA11pter-q13 is known to harbour a cluster of imprinted genes: IGF2, INS2, H19, MAH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, Tapa1./CD81, Orct12, Impt1 and Ip1. The cluster of imprinted genes located in HSA11pter-q13 is characterised by 8 maternally expressed genes: H19, MASH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, TAPA1/CD81, ORCTL2, IMPT1 and IPI, and two paternally expressed genes: IGF2 and INS. However, Johanson et al. (Genomics 25:682-690, 1995) and Reik et al. (Trends in Genetics, 13:330-334, 1997) show that the whereabouts of these loci in various animals are not clear. For example, the HSA11 and MMU7 loci do not correspond among each other, the MMU7 and the SSC2 loci do not correspond, whereas the HSA11

and SSC2 loci seem to correspond, and no guidance is given where one or more of, for example, the above-identified parentally expressed individual genes are localised on the three species' chromosomes.

**[0030]** Other domestic animals, such as cattle, sheep, poultry and fish, having similar regions in their genome harbouring such a cluster of imprinted genes or QTLs, the invention herewith provides use of these orthologous regions of other domestic animals in applying the phenomenon of parental imprinting in breeding programmes. In pigs, said cluster is mapped at around position 2p1.7 of chromosome 2; however, a method as provided by the invention employing (fragments of) said maternally or paternally expressed orthologous or homologous genes or QTLs is advantageously used in other animals as well as for breeding and selecting purposes. For example, a method is provided wherein said QTL is related to the potential muscle mass and/or fat deposition, preferably with limited effects on other traits such as meat quality and daily gain of the animal or wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) allele. Reik et al. (Trends in Genetics, 13:330-334, 1997) explain that this gene in humans is related to Beckwith-Wiedemann syndrome, an apparently parentally imprinted disease syndrome most commonly seen with human fetuses, where the gene has an important role in prenatal development. No relationship is shown or suggested with postnatal development relating to muscle development or fatness in (domestic) animals.

**[0031]** In a preferred embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7. In particular, the invention relates to the use of genetic markers for the telomeric end of pig chromosome 2p in marker selection (MAS) of a parentally imprinted Quantitative Trait Locus (QTL) affecting carcass yield and quality in pigs. Furthermore, the invention relates to the use of genetic markers associated with the IGF2 locus in MAS in pigs, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. In a preferred embodiment, the invention provides a QTL located at the distal tip of *Sus scrofa* chromosomes 2 with effects on various measurements of carcass quality and quantity, particularly muscle mass and fat deposition.

[0032] In a first experiment, a QTL mapping analysis was performed in a Wild Boar X Large White intercross counting 200 F<sub>2</sub> individuals. The F<sub>2</sub> animals were sacrificed at a live weight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are provided by Andersson et al.<sup>1</sup> and Andersson-Eklund et al.<sup>4</sup>

[0033] A QTL (without any significant effect on back-fat thickness) at an unspecified locus on the proximal end of chromosome 2 with moderate effect on muscle mass, and located about 30cM away from the parentally imprinted QTL reported here, was previously reported by the inventors; whereas the QTL as now provided has a very large effect, explaining at least 20-30% of variance, making the QTL of the present invention commercially very attractive, which is even more so because the present QTL is parentally imprinted. The marker map of chromosome 2p was improved as part of this invention by adding microsatellite markers in order to cover the entire chromosome arm. The following microsatellite markers were used: *Swc9*, *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map. Clear evidence for a QTL located at the very distal tip of 2p was obtained (Fig. 1; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population.

[0034] In a second experiment, QTL mapping was performed in a Piétrain X Large White intercross comprising 1125 F<sub>2</sub> offspring. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famous for their exceptional muscularity and leanness<sup>10</sup> (Figure 2), while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring growth performance (5), muscularity (6), fat deposition (6), and meat quality (4), were recorded on all F<sub>2</sub> offspring. In order to map QTL underlying the genetic differences

between these breeds, the inventors undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. The following microsatellite marker map was used to analyse chromosome 2; SW2443, SWC9 and SW2623, SWR2516- (0,20) -SWR783- (0,29) -SW240- (0,20) -SW776- (0,08) -S0010- (0, 04) -SW1695-(0,36) -SWR308. Analysis of pig chromosome 2 using a Maximum Likelihood multipoint algorithm revealed highly significant lodscores (up to 20) for three of the six phenotypes measuring muscularity (% lean cuts, % ham, % loin) and three of the six phenotypes measuring fat deposition (back-fat thickness (BFT), % backfat, % fat cuts) at the distal end of the short arm of chromosome 2 (Figure 1). Positive lodscores were obtained in the corresponding chromosome region for the remaining six muscularity and fatness phenotypes, however, not reaching the experiment-wise significance threshold ( $\alpha=5\%$ ). There was no evidence for an effect of the corresponding QTL on growth performance (including birth weight) or recorded meat quality measurements (data not shown). To confirm this finding, the remaining sample of 355 F<sub>2</sub> offspring was genotyped for the four most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for the three genotypic means as well as the residual variance. Evidence based on marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population.

**[0035]** These experiments, therefore, clearly indicated the existence of a QTL with a major effect on carcass quality and quantity on the telomeric end of pig chromosome arm 2p, the likely existence of an allelic series at this QTL with at least three alleles: Wild-Boar < Large White < Piétrain, and possibly more given the observed segregation within the Piétrain breed.

**[0036]** The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the *CRC* locus though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect

an one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL, when compared to the Wild Boar allele, was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits shows that a single causative locus is involved. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output.

**[0037]** In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7., wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele or a genomic area closely related thereto, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. The important role of IGF2 for prenatal development is well-documented from knockout mice as well as from its causative role in the human Beckwith-Wiedemann syndrome. This invention demonstrates an important role for the IGF2-region, also for postnatal development.

**[0038]** To show the role of IGF2, the inventors performed the following three experiments:

**[0039]** A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p.

**[0040]** A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800bp downstream of the stop codon; a sequence comparison revealed that this microsatellite was identical to a previously described anonymous microsatellite, *Swc9*<sup>6</sup>. This marker was used in the initial QTL mapping experiments and its location on the genetic map correspond with the most likely position of the QTL both in the Piétrain X Large White and in the Large White x Wild Boar pedigree.



**[0041]** Analysis of skeletal muscle and liver cDNA from 10-week old fetuses heterozygous for a nt241 (G-A) transversion in the second exon of the porcine IGF2 gene and SWC9 shows that the IGF2 gene is imprinted in these tissues in the pig as well and only expressed from the paternal allele.

**[0042]** Based on a published porcine adult liver cDNA sequence<sup>16</sup>, the inventors designed primer pairs allowing amplification of the entire IGF2 coding sequence with 222 bp of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced, indicating that the coding sequences are identical in both breeds and with the published sequence. However, a G→A transition was found in the leader sequence corresponding to exon 2 in man. Following conventional nomenclature, this polymorphism will be referred to as *nt241* (G-A). We developed a screening test for this single nucleotide polymorphism (SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, IGF2 was shown to colocalize with the SWC9 microsatellite marker ( $\theta=0\%$ ), therefore virtually coinciding with the most likely position of the QTL, and well within the 95% support interval for the QTL. Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3'UTR of the IGF2 gene.

**[0043]** As previously mentioned, the knowledge of this QTL provides a method for the selection of animals such as pigs with improved carcass merit. Different embodiments of the invention are envisaged, including: marker-assisted segregation analysis to identify the segregation of functionally distinct QTL alleles in the populations of interest; marker-assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy or selection intensity or by reducing the generation interval; and marker-assisted introgression (MAI) to efficiently transfer favourable QTL alleles from a donor to a recipient population, thereby enhancing genetic response in the recipient population. Implementation of embodiments of marker-assisted segregation analysis, selection (MAS) and introgression (MAI) can be performed using genetic markers that are linked to the QTL, genetic markers that are in linkage disequilibrium with the QTL, and the actual causal mutations within the QTL.

[0044] In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2p1.7., wherein said QTL is paternally expressed, i.e., is expressed from the paternal allele. In man and mouse, IGF2 is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9 shows that the same imprinting holds in the pig as well. Understanding the parent-of-origin effect characterising the QTL as provided by the invention now allows for its optimal use in breeding programmes. Indeed, today, half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele, causing considerable loss. Using a method as provided by the invention avoids this problem.

[0045] The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof comprising a parentally imprinted quantitative trait locus (QTL) or fragment thereof capable of being predominantly expressed by one parental allele. Having such a nucleic acid as provided by the invention available allows constructing transgenic animals wherein favourable genes are capable of being exclusively or predominantly expressed by one parental allele, thereby equipping the offspring of the animal homozygous for a desired trait with desired properties related to that parental allele that is expressed.

[0046] In a preferred embodiment, the invention provides an isolated and/or recombinant nucleic acid or fragment derived thereof comprising a synthetic parentally imprinted quantitative trait locus (QTL) or functional fragment thereof derived from at least one chromosome. Synthetic herein describes a parentally expressed QTL wherein various elements are combined that originate from distinct locations from the genome of one or more animals. The invention provides recombinant nucleic acid wherein sequences related to parental imprinting of one QTL are combined with sequences relating to genes or favourable alleles of a second QTL. Such a gene construct is favourably used to obtain transgenic animals wherein the second QTL has been equipped with paternal imprinting, as opposed to the inheritance pattern in the native animal from which the second QTL is derived. Such a second QTL can, for example, be derived from the same chromosome where the parental imprinting region is located, but can also be derived from a different chromosome from

the same or even a different species. In the pig, such a second QTL can, for example, be related to an oestrogen receptor (ESR)-gene (Rothschild et al., PNAS, 93, 201-201, 1996) or a FAT-QTL (Andersson, Science, 263, 1771-1774, 1994) for example, derived from another pig chromosome, such as chromosome 4. A second or further QTL can also be derived from another (domestic) animal or a human.

**[0047]** The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof at least partly corresponding to a QTL of a pig located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7 wherein said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele, preferably at least spanning a region between INS and H19, or preferably derived from a domestic pig, such as a Piétrain, Meishan, Duroc, Landrace or Large White, or from a Wild Boar. For example, a genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong, consistent signal on the terminal part of chromosome 2p. A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, *Swc9*. PGR primers were designed and the microsatellite (IGF2ms) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two among the eight Large White founders. IGF2ms was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each F<sub>2</sub> animal.

**[0048]** A linkage analysis using the intercross pedigree was carried out with IGF2ms and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. IGF2 was firmly assigned to 2p by highly significant lodscores (e.g. Z=89.0,  $\theta$ = 0.003 against *Swr2516*). Multipoint analyses, including previously typed chromosome 2 markers, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443*/*Swr2516*-0.3-IGF2-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed between *Sw2443* and *Swr2516*, and the suggested proximal location

of IGF2 in relation to these loci is based on a single recombinant giving a lodscore support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

**[0049]** The invention furthermore provides use of a nucleic acid or functional fragment derived thereof according to a method according to the invention. In a preferred embodiment, use of a method according to the invention is provided to select a breeding animal or animal destined for slaughter, or embryos or semen derived from these animals, for having desired genotypic or potential phenotypic properties. In particular, the invention provides such use wherein said properties are related to muscle mass and/or fat deposition. The QTL as provided by the invention may be exploited or used to improve, for example, lean meat content or back-fat thickness by marker-assisted selection within populations or by marker-assisted introgression of favourable alleles from one population to another. Examples of marker-assisted selection using the QTL as provided by the invention are use of marker-assisted segregation analysis with linked markers or with markers in disequilibrium to identify functionally distinct QTL alleles. Furthermore, identification of a causative mutation in the QTL is now possible, again leading to identify functionally distinct QTL alleles. Such functionally distinct QTL alleles located at the distal tip of chromosome 2p with large effects on skeletal muscle mass, the size of the heart, and on back-fat thickness are also provided by the invention. The observation of a similar QTL effect in a Large White x Wild Boar as well as in a Piétrain x Large White intercross provides proof of the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series as provided by the invention allows identifying causal polymorphisms which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found, i.e., Piétrain pigs are more muscular than Large White pigs that in turn have higher lean meat content than Wild Boars. The invention furthermore provides use of the alleles as provided by the invention within line selection or for marker-assisted introgression using linked markers, markers in disequilibrium or alleles comprising causative mutations.

[0050] The invention furthermore provides an animal selected by using a method according to the invention. For example, a pig characterised in being homozygous for an allele in a QTL located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7 can now be selected and is thus provided by the invention. Since said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele, it is possible to select promising pigs to be used for breeding or to be slaughtered. In particular, an animal according to the invention which is a male is provided. Such a male, or its sperm or an embryo derived thereof, can advantageously be used in breeding animals for creating breeding lines or for finally breeding animals destined for slaughter. In a preferred embodiment of such use as provided by the invention, a male, or its sperm, deliberately selected for being homozygous for an allele causing the extreme muscular hypertrophy and leanness, is used to produce offspring heterozygous for such an allele. Due to said allele's paternal expression, said offspring will also show the favourable traits, for example, related to muscle mass, even if the parent female has a different genetic background. Moreover, it is now possible to positively select the female(s) for having different traits, for example, related to fertility, without having a negative effect on the muscle mass trait that is inherited from the allele from the selected male. For example, earlier such males could occasionally be seen with Piétrain pigs but genetically it was not understood how to most profitably use these traits in breeding programmes.

[0051] Furthermore, the invention provides a transgenic animal, sperm and an embryo derived thereof, comprising a synthetic parentally imprinted QTL or functional fragment thereof as provided by the invention, i.e., it is provided by the invention to introduce a favourable recombinant allele; for example, to introduce the oestrogen receptor locus related to increased litter size of an animal homozygously in a parentally imprinted region of a grandparent animal (for example, the father of a hybrid sow if the region was paternally imprinted and the grandparent was a boar); to introduce a favourable fat-related allele or muscle mass-related recombinant allele in a paternally imprinted region, and so on. Recombinant alleles that are interesting or favourable from the maternal side are often the ones that have opposite effects to alleles from the paternal side. For example, in meat animals such as pigs, recombinant alleles linked with meat quality traits such as intramuscular fat or muscle mass could be fixed in the dam lines while recombinant alleles linked with reduced backfat

could be fixed in the sire lines. Other desirable combinations are, for example, fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

[0052] The invention is further explained in the detailed description without limiting the invention.

#### Example 1: Wild Boar x Large White intercrosses

#### Methods

[0053] Isolation of an IGF2 BAC clone and fluorescent *in situ* hybridisation (FISH). IGF2 primers (F:5'- GGCAAGTTCTTCCGCTAATGA-3' (SEQ. ID. NO: \_\_\_\_ ) and R:5' - GCACCGCAGAATTACGACAA-3' (SEQ. ID. NO: \_\_\_\_)) for PCR amplification of a part of the last exon and 3'UTR were designed on the basis of a porcine IGF2 cDNA sequence (GenBank X56094). The primers were used to screen a porcine BAC library and the clone 253G10 was isolated. Crude BAC DNA was prepared as described<sup>24</sup>. The BAC DNA was linearized with *EcoRV* and purified with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two days at room temperature and then kept at -20°C until use. FISH analysis was carried out as previously described<sup>25</sup>. The final concentration of the probe in the hybridisation mix was 10 ng/μl. Repetitive sequences were suppressed with standard concentrations of porcine genomic DNA. After post-hybridisation washing, the biotinylated probe was detected with two layers of avidin-FITC (Vector A-2011). The chromosomes were counterstained with 0.3 mg/ml DAPI (4, 6-Diamino-2-phenylindole; Sigma D9542), which produced a G-banding like pattern. No posthybridisation banding was needed, since chromosome 2 is easily recognized without banding. A total of 20 metaphase spreads were examined under an Olympus BX-60 fluorescence microscope connected to an IMAC-CCD S30 video camera and equipped with an ISIS 1.65 (Metasystems) software.

Sequence, microsatellite, and linkage analysis.

[0054] About two  $\mu\text{g}$  of linearized and purified BAC DNA was used for direct sequencing with 20 pmoles of primers and BigDye Terminator chemistry (Perkin Elmer, USA). DNA sequencing was done from the 3' end of the last exon towards the 3' end of the UTR until a microsatellite was detected. A primer set (F: 5'-GTTTCTCCTGTACCCACACGCATCCC-3' (SEQ. ID. NO. \_\_\_\_)) and R: 5'-Fluorescein-CTACAAGCTGGGCTCAGGG-3' (SEQ. ID. NO. \_\_\_\_)) was designed for the amplification of the IGF2 microsatellite which is about 250 bp long and located approximately 800 bp downstream from the stop codon. The microsatellite was PCR amplified using fluorescently labeled primers and the genotyping was carried out using an ABI377 sequencer and the GeneScan/Genotyper softwares (Perkin Elmer, USA). Two-point and multipoint linkage analyses were done with the Cri-Map software<sup>26</sup>.

Animals and phenotypic data.

[0055] The intercross pedigree comprised two European Wild Boar males and eight Large White females, 4 F<sub>1</sub> males and 22 F<sub>1</sub> females, and 200 F<sub>2</sub> progeny<sup>1</sup>. The F<sub>2</sub> animals were sacrificed at a live weight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits is provided by Andersson *et al.*<sup>1</sup> and Andersson-Eklund *et al.*<sup>4</sup>

Statistical analysis.

[0056] Interval mapping for the presence of QTL was carried out using a least squares method developed for the analysis of crosses between outbred lines<sup>27</sup>. The method is based on the assumption that the two divergent lines are fixed for alternative QTL alleles. There are four possible genotypes in the F<sub>2</sub> generation with regards to the grandparental origin of the alleles at each locus. This makes it possible to fit three effects: additive, dominance, and imprinting<sup>2</sup>. The latter is

estimated as the difference between the two types of heterozygotes, the one receiving the Wild Boar allele through an F<sub>1</sub> sire and the one receiving it from an F<sub>1</sub> dam. An F-ratio was calculated using this model (with 3 d.f.) versus a reduced model without a QTL effect for each cM of chromosome 2. The most likely position of a QTL was obtained as the location giving the highest F-ratio. Genome-wise significant thresholds were obtained empirically by a permutation test<sup>28</sup> as described<sup>2</sup>. The QTL model including an imprinting effect was compared with a model without imprinting (with 1 d.f.) to test whether the imprinting effect was significant.

[0057] The statistical models also included the fixed effects and covariants that were relevant for the respective traits; see Andersson-Eklund *et al.*<sup>4</sup> for a more detailed description of the statistical models used. Family was included to account for background genetic effects and maternal effects. Carcass weight was included as a covariant to discern QTL effects on correlated traits, which means that all results concerning body composition were compared at equal weights. Least-squares means for each genotype class at the IGF2 locus were estimated with a single point analysis using Procedure GLM of SAS<sup>29</sup>; the model included the same fixed effects and covariants as used in the interval mapping analyses. The QTL shows a clear parent of origin-specific expression and the map position coincides with that of the insulin-like growth factor II gene (IGF2), indicating IGF2 as the causative gene. A highly significant segregation distortion (excess of Wild Boar-derived alleles) was also observed at this locus. The results demonstrate an important effect of the IGF2 region on postnatal development and it is possible that the presence of a paternally expressed IGF2-linked QTL in humans and in rodent model organisms has so far been overlooked due to experimental design or statistical treatment of data. The study has also important implications for quantitative genetics theory and practical pig breeding.

[0058] IGF2 was identified as a positional candidate gene for this QTL due to the observed similarity between pig chromosome 2p and human chromosome 11p. A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong, consistent signal on the terminal part of chromosome 2p (Fig. 1). A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite



was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, *Swc9*<sup>6</sup>. PCR primers were designed and the microsatellite (IGF2ms) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two among the eight Large White founders. IGF2ms was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each F<sub>2</sub> animal.

[0059] A linkage analysis using the intercross pedigree was carried out with IGF2ms and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. IGF2 was firmly assigned to 2p by highly significant lodscores (e.g.,  $Z=89.0$ ,  $\theta=0.003$  against *Swr2516*). Multipoint analyses, including previously typed chromosome 2 markers<sup>8</sup>, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443*/*Swr2516*-0.3-IGF2-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed between *Sw2443* and *Swr2516*, and the suggested proximal location of IGF2 in relation to these loci is based on a single recombinant giving a lodscore support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

[0060] QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map using a statistical model testing for the possible presence of an imprinting effect as expected for IGF2. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits strongly suggests a single causative locus. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output. The clear paternal expression of this QTL is illustrated

by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). It is worth noticing though that there was a non-significant trend towards less extreme values for the two heterozygous classes, in particular for the estimated effect on the area of longissimus dorsi. This may be due to chance, but could have a biological explanation, e.g., that there is some expression of the maternally inherited allele or that there is a linked, non-imprinted QTL with minor effects on the traits in question.

[0061] The IGF2-linked QTL and the *FAT1* QTL on chromosome 4 1, 9 are by far the two loci with the largest effect on body composition and fatness segregating in this Wild Boar intercross. The IGF2 QTL controls primarily muscle mass whereas *FAT1* has major effects on fat deposition including abdominal fat, a trait that was not affected by the IGF2 QTL (Fig. 2). No significant interaction between the two loci was indicated and they control a very large proportion of the residual phenotypic variance in the F<sub>2</sub> generation. A model including both QTLs explains 33.1% of the variance for percentage of lean meat in ham, 31.3% for the percentage of lean meat plus bone in back, and 26.2% for average backfat depth (compare with a model including only chromosome 2 effects, Table 1). The two QTLs must have played a major role in the response during selection for lean growth and muscle mass in the Large White domestic pig.

[0062] A highly significant segregation distortion was observed in the IGF2 region (excess of Wild Boar-derived alleles) as shown in Table 1 ( $\chi^2=11.7$ , d.f.=2;  $P=0.003$ ). The frequency of Wild Boar-derived IGF2 alleles was 59% in contrast to the expected 50% and there was twice as many “Wild Boar” as “Large White” homozygotes. This deviation was observed with all three loci at the distal tip and is thus not due to typing errors. The effect was also observed with other loci but the degree of distortion decreased as a function of the distance to the distal tip of the chromosome. Blood samples for DNA preparation were collected at 12 weeks of age and we are convinced that the deviation from expected Mendelian ratios was present at birth as the number of animals lost prior to blood sampling was not sufficient enough to cause a deviation of this magnitude. No other of the more than 250 loci analysed in this pedigree show such a marked segregation distortion (L. Andersson, unpublished). The segregation distortion did not show an imprinting effect, as the frequencies of the two reciprocal types of heterozygotes were identical (Table 1). This does not exclude the possibility that the QTL effects and the segregation distortion are controlled by the same

locus. The segregation distortion may be due to meiotic drive favouring the paternally expressed allele during gametogenesis, as the F<sub>1</sub> parents were all sired by Wild Boar males. Another possibility is that the segregation distortion may be due to codominant expression of the maternal and paternal allele in some tissues and/or during a critical period of embryo development. Biallelic IGF2 expression has been reported to occur to some extent during human development<sup>10, 11</sup> and, interestingly, a strong influence of the parental species background on IGF2 expression was recently found in a cross between *Mus musculus* and *Mus spretus*<sup>12</sup>. It is also interesting that a VNTR polymorphism at the insulin gene, which is very closely linked to IGF2, is associated with size at birth in humans<sup>13</sup>. It is possible that the IGF2-linked QTL in pigs has a minor effect on birth weight, but in our data it was far from significant (Fig. 2) and there was no indication of an imprinting effect.

**[0063]** This study is an advance in the general knowledge concerning the biological importance of the IGF2 locus. The important role of IGF2 for prenatal development is well-documented from knock-out mice<sup>14</sup> as well as from its causative role in the human Beckwith-Wiedemann syndrome<sup>15</sup>. This study demonstrates an important role for the IGF2-region also for postnatal development. It should be stressed that our intercross between outbred populations is particularly powerful to detect QTL with a parent of origin-specific effect on a multifactorial trait. This is because multiple alleles (or haplotypes) are segregating and we could deduce whether a heterozygous F<sub>2</sub> animal received the Wild Boar allele from the F<sub>1</sub> male or female. It is quite possible that the segregation of a paternally expressed IGF2-linked QTL affecting a trait like obesity has been overlooked in human studies or in intercrosses between inbred rodent populations because of experimental design or statistical treatment of data. An imprinting effect cannot be detected in an intercross between two inbred lines as only two alleles are segregating at each locus. Our result has, therefore, significant bearings on the future analysis of the association between genetic polymorphism in the Insulin-IGF2 region and Type I diabetes<sup>16</sup>, obesity<sup>17</sup>, and variation in birth weight<sup>13</sup> in humans, as well as for the genetic dissection of complex traits using inbred rodent models. A major impetus for generating an intercross between the domestic pig and its wild ancestor was to explore the possibilities to map and identify major loci that have responded to selection. We have now showed that two single QTLs on chromosome 2 (this study) and 4<sup>1, 2</sup> explain as much as one third of the phenotypic variance for lean meat content in the F<sub>2</sub> generation. This is a gross deviation from the

underlying assumption in the classical infinitesimal model in quantitative genetics theory, namely, that quantitative traits are controlled by an infinite number of loci, each with an infinitesimal effect. If a large proportion of the genetic difference between two divergent populations (e.g., Wild Boar and Large White) is controlled by a few loci, one would assume that selection would quickly fix QTL alleles with large effects leading to a selection plateau. However, this is not the experience in animal breeding programmes or selection experiments where good, persistent, long-term selection responses are generally obtained, provided that the effective population size is reasonably large<sup>18</sup>. A possible explanation for this paradox is that QTL alleles controlling a large proportion of genetic differences between two populations may be due to several consecutive mutations; this may be mutations in the same gene or at several closely linked genes affecting the same trait. It has been argued that new mutations contribute substantially to long-term selection responses<sup>19</sup>, but the genomic distribution of such mutations is unknown.

**[0064]** The search for a single causative mutation is the paradigm regarding the analysis of genetic defects in mice and monogenic disorders in humans. We propose that this may not be the case for loci that have been under selection for a large number of generations in domestic animals, crops, or natural populations. This hypothesis predicts the presence of multiple alleles at major QTL. It gains some support from our recent characterisation of porcine coat color variation. We have found that both the alleles for dominant white color and for black-spotting differ from the corresponding wild-type alleles by at least two consecutive mutations with phenotypic effects at the *KIT* and *MC1R* loci, respectively<sup>20, 21</sup>. In this context, it is highly interesting that in the accompanying example we have identified a third allele at the IGF2-linked QTL. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found, i.e., Piétrain pigs are more muscular than Large White pigs, which in turn have a higher lean meat content than Wild Boars.

**[0065]** There are good reasons to decide that IGF2 is the causative gene for the now reported QTL. Firstly, there is a perfect agreement in map localization (Fig. 2). Secondly, it has been shown that IGF2 is paternally expressed in mice, humans, and now in pigs, like the QTL. There are several other imprinted genes in the near vicinity of IGF2 in mice and humans (*Mash2*, *INS2*, *H19*, *KVLQT1*, *TAPAI/CD81*, and *CDKNIC/p57<sup>KIP2</sup>*) but only IGF2 is paternally expressed in adult tissues<sup>22</sup>. We believe that this locus provides a unique opportunity for molecular characterisation of a QTL. The

clear paternal expression can be used to exclude genes that do not show this mode of inheritance. Moreover, the presence of an allelic series should facilitate the difficult distinction between causative mutations and linked neutral polymorphism. We have already shown that there is no difference in coding sequence between IGF2 alleles from Piétrain and Large White pigs suggesting that the causative mutations occur in regulatory sequences. An obvious step is to sequence the entire IGF2 gene and its multiple promoters from the three populations. The recent report that a VNTR polymorphism in the promoter region of the insulin (*INS*) gene affects IGF2 expression<sup>23</sup> suggests that the causative mutations may be at a considerable distance from the IGF2 coding sequence.

[0066] The results have several important implications for the pig breeding industry. They show that genetic imprinting is not an esoteric academic question but needs to be considered in practical breeding programmes. The detection of three different alleles in Wild Boar, Large White, and Piétrain populations indicates that further alleles at the IGF2-linked QTL segregate within commercial populations. The paternal expression of the QTL facilitates its detection using large paternal half-sib families as the female contribution can be ignored. The QTL is exploited to improve lean meat content by marker-assisted selection within populations or by marker-assisted introgression of favourable alleles from one population to another.

## Example 2: Piétrain x Large White intercrosses

### Methods

[0067] *Pedigree material:* The pedigree material utilised to map QTL was selected from a previously described Piétrain x Large White F2 pedigree comprising > 1,800 individuals<sup>6,7</sup>. To assemble this F2 material, 27 Piétrain boars were mated to 20 Large White sows to generate an F1 generation comprising 456 individuals. 31 F1 boars were mated to unrelated 82 F1 sows from 1984 to 1989, yielding a total of 1862 F2 offspring. F1 boars were mated on average to 7 females, and F1 sows to an average of 2, 7 males. Average offspring per boar was 60 and per sow was 23.

[0068] *Phenotypic information:* (i) *Data collection:* A total of 21 distinct phenotypes were recorded in the F2 generation<sup>6,7</sup>. These included:

- five growth traits: birth weight (g), weaning weight (Kg), grower weight (Kg), finisher weight (Kg) and average daily gain (ADG; Kg/day; grower to finisher period);
- two body proportion measurements: carcass length (cm); and a conformation score (0 to 10 scale; ref.6);
- ten measurements of carcass composition obtained by dissection of the chilled carcasses 24 hours after slaughter. These include measurements of muscularity: % ham (weight hams/carcass weight), % loin (weight loin/carcass weight), % shoulder (weight shoulder/carcass weight), % lean cuts (% ham + % loin + % shoulder); and measurements of fatness: average back-fat thickness (BFT; cm), % backfat (weight backfat/carcass weight), % belly (weight belly/carcass weight), % leaf fat (weight leaf fat/carcass weight), % jowl (weight jowl/carcass weight), and “% fat cuts” (% backfat + % belly + % leaf fat + % jowl).
- four meat quality measurements: pH<sub>LD1</sub> (*Longissimus dorsi* 1 hour after slaughter), pH<sub>LD24</sub> (*Longissimus dorsi* 24 hours after slaughter), pH<sub>GL</sub> (*Gracilis* 1 hour after slaughter) and pH<sub>G24</sub> (*Gracilis* 24 hours after slaughter). (ii) *Data processing*: Individual phenotypes were preadjusted for fixed effects (sire, dam, CRC genotype, sex, year-season, parity) and covariants (litter size, birth weight, weaning weight, grower weight, finisher weight) that proved to significantly affect the corresponding trait. Variables included in the model were selected by stepwise regression.

[0069] *Marker genotyping*: Primer pairs utilised for PCR amplification of microsatellite markers are as described<sup>19</sup>. Marker genotyping was performed as previously described<sup>20</sup>. Genotypes at the *CRC* and *MyoD* loci were determined using conventional methods as described<sup>1,12</sup>. The LAR test for the IGF2 SNP was developed according to Baron et al.<sup>21</sup> using a primer pair for PCR amplification (5'-CCCCTGAACTTGAGGACGAGCAGCC-3' (SEQ. ID. NO. \_\_\_\_); 5'-ATCGCTGTGGGCTGGGTGGGCTGCC-3') (SEQ. ID. NO. \_\_\_\_)) and a set of three primers for the LAR step (5'-FAM- CGCCCCAGCTGCCCCCAG-3' (SEQ. ID. NO. \_\_\_\_); 5' -HEX- CGCCCCAGCTGCCCCCAA-3' (SEQ. ID. NO. \_\_\_\_); 5' -CCTGAGCTGCAGCAGGCCAG-3') (SEQ. ID. NO. \_\_\_\_).

[0070] *Map construction*: Marker maps were constructed using the TWOPOINT, BUILD and CHROMPIC options of the CRIMAP package<sup>22</sup>. To allow utilisation of this package, full-sib families related via the boar or sow were disconnected and treated independently. By doing so, some

potentially usable information was neglected, yielding, however, unbiased estimates of recombination rates.

[0071] *QTL mapping: (i) Mapping Mendelian QTL:* Conventional QTL mapping was performed using a multipoint maximum likelihood method. The applied model assumed one segregating QTL per chromosome and fixation of alternate QTL alleles in the respective parental lines, Piétrain (P) and Large White (LW). A specific analysis programme had to be developed to account for the missing genotypes of the parental generation, resulting in the fact that the parental origin of the F1 chromosomes could not be determined. Using a typical “interval mapping” strategy, a hypothetical QTL was moved along the marker map using user-defined steps. At each position, the likelihood ( $L$ ) of the pedigree data was computed as:

$$L = \sum_{\varphi=1}^{2^r} \prod_{i=1}^n \sum_{G=1}^4 (P(G|M_i, \theta, \varphi) P(y_i|G))$$

$P$  or right chromosome  $P$ ), there is a total of  $2^r$  combinations for  $r$  F1 parents.

$$\prod_{i=1}^n n \text{ F2}$$

$$\sum_{G=1}^4 \text{ } i\text{th F2 offspring, over the four possible QTL genotypes:}$$

$P/P$ ,  $P/LW$ ,  $LW/P$  and  $LW/LW$

$P(G|M_i, \theta, \varphi)$ : the marker genotype of the  $i$ th F2 offspring and its F1 parents, (ii) the vector of recombination rates between adjacent markers and between the hypothetical QTL and its flanking markers, and (iii)  $\theta$  the considered marker-QTL phase combination of the F1 parents.

Recombination rates and marker linkage phase of F1 parents are assumed to be known when computing this probability. Both were determined using CRIMAP in the map construction phase (see above).

$P(y_i|G)y_i)$  of offspring  $i$ , given the QTL genotype under consideration. This probability is computed from the normal density function:

$$P(y_i|G) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(y_i - \mu_G)^2}{2\sigma^2}}$$

$\mu_G$  is the phenotypic mean of the considered QTL genotype (PP, PL, LP or LL) and  $\sigma^2$  the residual variance.  $\sigma^2$  was considered to be the same for the four QTL genotypic classes. The values of  $\mu_{PP}$ ,  $\mu_{PL} = \mu_{LP}$ ,  $\mu_{LL}$  and  $\sigma^2$  maximizing  $L$  were determined using the GEMINI optimisation routine<sup>23</sup>. The likelihood obtained under this alternative  $H_1$  hypothesis was compared with the likelihood obtained under the null hypothesis  $H_0$  of no QTL, in which the phenotypic means of the four QTL genotypic classes were forced to be identical. The difference between the logarithms of the corresponding likelihoods yields a lodscore measuring the evidence in favour of a QTL at the corresponding map position.

(ii) *Significance thresholds:* Following Lander & Botstein<sup>24</sup>, lodscore thresholds ( $T$ ) associated with a chosen genome-wise significance level were computed such that:

$$\alpha = (C + 9.21GT)\chi^2_2(4.6T)$$

$C$  corresponds to the number of chromosomes (= 19),  $G$  corresponds to the length of the genome in Morgans (= 29), and  $\chi^2_2(4.6T)$  denotes one minus the cumulative distribution function of the chi-squared distribution with 2 d.f. Single point 21n(LR) were assumed to be distributed as a chi-squared distribution with two degrees of freedom, as we were fitting both an additive and dominance component. To account for the fact that we were analysing multiple traits, significance levels were adjusted by applying a Bonferoni correction corresponding to the effective number of independent traits that were analysed. This effective number was estimated at 16 following the approach described



by Spelman et al.<sup>25</sup>. Altogether, this allowed us to set the lodscore threshold associated with an experiment—wise significance level of 5% at 5.8. When attempting to confirm the identified QTL in an independent sample, the same approach was used, however, setting C at 1, G at 25cM and correcting for the analysis of 4.5 independent traits (as only six traits were analysed in this sample). This yielded a lodscore threshold associated with a Type I error of 5% of 2.

(iii) *Testing for an imprinted QTL*: To test for an imprinted QTL, we assumed that only the QTL alleles transmitted by the parent of a given sex would have an effect on phenotype, the QTL alleles transmitted by the other parent being “neutral”. The likelihood of the pedigree data under this hypothesis was computed using equation 1. To compute  $P(y_i | G)$ , however, the phenotypic means of the four QTL genotypes were set at  $\mu_{PP} = \mu_{PL} = \mu_P$  and  $\mu_{LP} = \mu_{LL} = \mu_L$  to test for a QTL for which the paternal allele only is expressed, and  $\mu_{PP} = \mu_{LP} = \mu_P$  and  $\mu_{PL} = \mu_{LL} = \mu_L$  to test for a QTL for which the maternal allele only is expressed. It is assumed in this notation that the first subscript refers to the paternal allele, the second subscript to the maternal allele.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  testing the presence of a Mendelian QTL,  $H_2$  testing the presence of a paternally expressed QTL, and  $H_3$  testing the presence of a maternally expressed QTL.

[0072] *RT-PCR*: Total RNA was extracted from skeletal muscle according to Chirgwin et al.<sup>26</sup> RT-PCR was performed using the Gene-Amp RNA PCR Kit (Perkin-Elmer) The PCR products were purified using QiaQuick PCR Purification kit (Qiagen) and sequenced using Dye terminator Cycle Sequencing Ready Reaction (Perkin Elmer) and an ABI373 automatic sequencer. In example 2 we report the identification of a QTL with major effect on muscle mass and fat deposition mapping to porcine 2p1.7. The QTL shows clear evidence for parental imprinting strongly suggesting the involvement of the IGF2 locus.

[0073] A Piétrain X Large White intercross comprising 1125  $F_2$  offspring was generated as described<sup>6, 7</sup>. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrain are famed for their exceptional muscularity and leanness<sup>8</sup> (Figure 2), while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring (i) growth performance (5), (ii) muscularity (6), (iii) fat deposition (6), and (iv) meat quality (4) were recorded on all  $F_2$  offspring.

[0074] In order to map QTL underlying the genetic differences between these breeds, we undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. Analysis of pig chromosome 2 using a ML multipoint algorithm revealed highly significant lodscores (up to 20) for six of the 12 phenotypes measuring muscularity and fat deposition at the distal end of the short arm of chromosome 2 (Figure 3a). Positive lodscores were obtained for the remaining six phenotypes, however, not reaching the genome-wide significance threshold (= 5%). To confirm this finding, the remaining sample of 355 F<sub>2</sub> offspring was genotyped for the five most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for the three genotypic means as well as the corresponding residual variance.

[0075] Bidirectional chromosome painting establishes a correspondence between SSC2p and HSA1pter-q13<sup>9, 10</sup>. At least two serious candidate genes map to this region in man: the myogenic basic helix-loop-helix factor, *MyoD*, maps to HSA11p15.4, while IGF2 maps to HSA11p15.5. *MyoD* is a well-known key regulator of myogenesis and is one of the first myogenic markers to be switched on during development<sup>11</sup>. A previously described amplified sequence polymorphism in the porcine *MyoD* gene<sup>12</sup> proved to segregate in our F<sub>2</sub> material, which was entirely genotyped for this marker. Linkage analysis positioned the *MyoD* gene in the SW240-SW776 (odds > 1000) interval, therefore, well outside the lod-2 drop off support interval for the QTL (figure 1). IGF2 is known to enhance both proliferation and differentiation of myoblasts *in vitro*<sup>13</sup> and to cause a muscular hypertrophy when overexpressed *in vivo*. Based on a published porcine adult liver cDNA sequence<sup>14</sup>, we designed primer pairs allowing us to amplify the entire IGF2 coding sequence with 222 bp of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced, indicating that the coding sequences were identical in both breeds and with the published sequence. However, a G A transition was found in the leader sequence corresponding to exon 2 in man (Figure 4). We developed a screening test for this single nucleotide polymorphism (SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, IGF2 was shown to colocalize with the SWC9 microsatellite marker (= 0%), therefore, located at approximately 2 centimorgan from the most likely position of the QTL

and well within the 95% support interval for the QTL (figure 1). Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3' UTR of the IGF2 gene. Combined with available comparative mapping data for the PGA and FSH loci, these results suggest the occurrence of an interstitial inversion of a chromosome segment containing *MyoD*, but not IGF2 which has remained telomeric in both species.

[0076] IGF2, therefore, appeared as a strong positional allele having the observed QTL effect. In man and mouse, IGF2 is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues<sup>15</sup>. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9 shows that the same imprinting holds in this tissue in the pig as well (Figure 4). Therefore, if IGF2 were responsible for the observed effect, and knowing that only the paternal IGF2 allele is expressed, one can predict that (i) the paternal allele transmitted by F1 boars (P or LW) would have an effect on the phenotype of F2 offspring, (ii) the maternal allele transmitted by F1 sows (P or LW) would have no effect on phenotype of F2 offspring, and (iii) the likelihood of the data would be superior under a model of a bimodal (1:1) F2 population sorted by inherited paternal allele when compared to a conventional "Mendelian" model of a trimodal (1:2:1) F2 population. The QTL mapping programmes were adapted in order to allow testing of the corresponding hypotheses.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL.

[0077] Figure 3 summarizes the obtained results. Figures 3a, 3b and 3c respectively show the lodscore curves corresponding to  $\log_{10} (H_2/H_0)$ ,  $\log_{10} (H_3/H_0)$  and  $\log_{10} (H_2/H_1)$ . It can be seen that very significant lodscores are obtained when testing for the presence of a paternally expressed QTL, while there is no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. Also, the hypothesis of a paternally expressed QTL is significantly more likely ( $\log_{10} (H_2/H_1) > 3$ ) than the hypothesis of a "Mendelian" QTL for all examined traits. The fact that the same tendency is observed for all traits indicates that it is likely the same imprinted gene that is responsible for the effects observed on the different traits. Table 2 reports the ML phenotypic means for the F2 offspring sorted by inherited paternal QTL allele. Note that when performing the analysis under a model of a mendelian QTL, the Piétrain and Large White QTL alleles appeared to

behave in an additive fashion, the heterozygous genotype exhibiting a phenotypic mean corresponding exactly to the midpoint between the two homozygous genotypes. This is exactly what one would predict when dealing with an imprinted QTL as half of the heterozygous offspring are expected to have inherited the P allele from their sire, the other half the LW allele.

[0078] These data, therefore, confirmed our hypothesis of the involvement of an imprinted gene expressed exclusively from the paternal allele. The fact that the identified chromosomal segment coincides precisely with an imprinted domain documented in man and mice strongly implicates the orthologous region in pigs. At least seven imprinted genes mapping to this domain have been documented (IGF2, Ins2, H19, Mash2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1 and TDAG51) (ref. 15 and Andrew Feinberg, personal communication). Amongst these, only IGF2 and Ins2 are paternally expressed. While we cannot exclude that the observed QTL effect is due to an as of yet unidentified imprinted gene in this region, its reported effects on myogenesis *in vitro* and *in vivo*<sup>13</sup> strongly implicate IGF2. Particularly the muscular hypertrophy observed in transgenic mice overexpressing IGF2 from a muscle-specific promotor are in support of this hypothesis (Nadia Rosenthal, personal communication). Note that allelic variants of the *INS* VNTR have recently been shown to be associated with size at birth in man<sup>16</sup>, and that the same VNTR has been shown to affect the level of IGF2 expression<sup>17</sup>.

[0079] The observation of the same QTL effect in a Large White x Wild Boar intercross indicates the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series might be invaluable in identifying the causal polymorphisms which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations.

[0080] The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the *CRC* locus<sup>6,7</sup> though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. Understanding the parent-of-origin effect characterising this locus will allow for its optimal use in breeding programmes.

Indeed, today, half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing considerable loss.

**[0081]** The QTL described in this work is the second example of a gene affecting muscle development in livestock species that exhibits a non-mendelian inheritance pattern. Indeed, we have previously shown that the callipyge locus (related to the qualitative trait wherein muscles are doubled) is characterised by polar overdominance in which only the heterozygous individuals that inherit the CLPG mutation from their sire express the double-muscling phenotype<sup>5</sup>. This demonstrates that parent-of-origin effects affecting genes underlying production traits in livestock might be relatively common.

### Example 3:

Generating a reference sequence of IGF2 and flanking loci in the pig.

**[0082]** The invention provides an imprinted QTL with a major effect on muscle mass mapping to the IGF2 locus in the pig, and use of the QTL as a tool in marker-assisted selection. To fine tune this tool for marker-assisted selection, as well as to further identify a causal mutation, we have further generated a reference sequence encompassing the entire porcine IGF2 sequence as well as that from flanking genes.

**[0083]** To achieve this, we screened a porcine BAC library with IGF2 probes and identified two BACs. BAC-PIGF2-1 proved to contain the INS and IGF2 genes, while BAC-PIGF2-2 proved to contain the IGF2 and H19 genes. The NotI map as well as the relative position of the two BACs is shown in Figure 5. BAC-PIGF2-1 was shotgun sequenced using standard procedures and automatic sequencers. The resulting sequences were assembled using standard software yielding a total of 115 contigs. The corresponding sequences are reported in figure 6. Similarity searches were performed between the porcine contigs and the orthologous sequences in humans. Significant homologies were detected for 18 contigs and are reported in Figure 7.

**[0084]** For BAC-PIGF2-2, the 24 Kb NotI fragment not present in BACPIGF2-1 was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

Resulting sequences were assembled using the Phred-Phrap-Consed programme suit, yielding seven distinct contigs (figure 8). The contig sequences were aligned with the corresponding orthologous human sequences using the compare and dotplot programmes of the GCG suite. Figure 9 summarizes the corresponding results.

Example 4: Identification of DNA sequence polymorphisms in the IGF2 and flanking loci.

[0085] Based on the reference sequence obtained as described in Example 1, we resequenced part of the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals, allowing identification of DNA sequence polymorphisms such as reported in figure 10.

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Table 1 Summary of QTL analysis for pig chromosome 2 in a Wild Boar/Large White intercross<sup>1</sup>

Trait	$L^P/L^M$	F ratio <sup>2</sup> <i>QTL</i>	Imprintin <i>g</i>	Map position <sup>3</sup>	Percent of $F_2$ variance <sup>4</sup>	Least squares means <sup>5</sup> $W^P/W^M$	$W^P/L^M$	$L^P/W^M$
<u>Body composition traits</u>								
Lean meat in ham, %		24.4***	19.1***	0	30.6	63.6 <sup>a</sup>	n=43	n=30
Lean meat mass in ham, kg		18.1***	16.8***	1	24.3	4.69 <sup>a</sup>	66.4 <sup>b</sup>	67.3 <sup>b</sup>
Lean meat + bone in back, %		12.2**	9.6**	0	17.4	66.3 <sup>a</sup>	4.94 <sup>b</sup>	5.02 <sup>b</sup>
Longissimus muscle area, cm <sup>2</sup>		10.3**	4.8*	1	15.4	31.9 <sup>a</sup>	69.3 <sup>b</sup>	70.8 <sup>b</sup>
<u>Fatness traits</u>								
Average backfat depth, mm		7.1*	8.7**	0	10.4	27.2 <sup>a</sup>	25.5 <sup>b</sup>	24.7 <sup>b</sup>
<u>Weight of internal organs</u>								
Heart, gram		9.7**	11.4***	0	14.4	226 <sup>a</sup>	238 <sup>b</sup>	244 <sup>b</sup>
<u>Meat quality traits</u>								
Reflectance value, EEL		5.7	6.1*	1	8.1	18.6 <sup>a</sup>	21.8 <sup>b</sup>	19.7 <sup>a</sup>

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

**Table 1, continued**

<sup>1</sup>Only the traits for which the QTL peak was in the IGF2 region (0-10 cM) and the test statistic reaching the nominal significance threshold of  $F=3.9$  are included.

<sup>2</sup>"*QTL*" is the test statistic for the presence of a QTL under a genetic model with additive, dominance, and imprinting effects (3 d.f.) while "Imprinting" is the test statistic for the presence of an imprinting effect (1 d.f.), both obtained at the position of the QTL peak. Genome-wise significance thresholds, estimated by permutation, were used for the QTL test while nominal significance thresholds were used for the Imprinting test.

<sup>3</sup>1n cM from the distal end of 2p; IGF2 is located at 0.3 cM.

<sup>4</sup>The reduction in the residual variance of the  $F_2$  population effected by inclusion of an imprinted QTL at the given position.

<sup>5</sup>Means and standard errors estimated at the IGF2 locus by classifying the genotypes according to the population and parent of origin of each allele. *W* and *L* represent alleles derived from the Wild Boar and Large White founders, respectively; superscripts *P* and *M* represent a paternal and maternal origin, respectively. Figures with different letters (superscript a or b) are significantly different at least at the 5% level, and most of them are different at the 1% or 0.1% level.



Table 2 Maximum likelihood phenotypic means for the different F2 genotypes estimated under (i) a model of a mendelian QTL, and (ii) a model assuming an imprinted QTL.

Traits	Mendelian QTL				Imprinted QTL		
	$\mu_{LW/LW}$	$\mu_{LW/P}$	$\mu_{P/P}$	R	$\mu_{PAT/LW}$	$\mu_{PAT/P}$	R
BFT (cm)	2.98	2.84	2.64	0.27	2.94	2.70	0.27
% ham	21.10	21.56	22.15	0.83	21.23	21.95	0.83
% loin	24.96	25.53	26.46	0.91	25.12	26.14	0.93
% lean cuts	65.02	65.96	67.60	1.65	65.23	67.05	1.67
% backfat	6.56	6.02	5.33	0.85	6.43	5.56	0.85
% fat cuts	28.92	27.68	26.66	1.46	28.54	26.99	1.49



## **APPENDIX B**

**(VERSION OF SUBSTITUTE SPECIFICATION EXCLUDING CLAIMS  
WITH MARKINGS TO SHOW CHANGES MADE)**

**(Serial No. 09/868,732)**



PATENT  
Attorney Docket 4951US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: \_\_\_\_\_

Date of Deposit with USPS: \_\_\_\_\_

Person making Deposit: \_\_\_\_\_

APPLICATION FOR LETTERS PATENT

for

**SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS**

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## SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS

### TECHNICAL FIELD

[0001] The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition.

### BACKGROUND

[0002] Breeding schemes for domestic animals have so far focused on farm performance traits and carcass quality. This has resulted in substantial improvements in traits like reproductive success, milk production, lean/fat ratio, prolificacy, growth rate and feed efficiency. Relatively simple performance test data have been the basis for these improvements, and selected traits were assumed to be influenced by a large number of genes, each of small effect (the infinitesimal gene model). There are now some important changes occurring in this area. First, the breeding goal of some breeding organisations has begun to include meat quality attributes in addition to the “traditional” production traits. [Secondly]Second, evidence is accumulating that current and new breeding goal traits may involve relatively large effects (known as major genes), as opposed to the infinitesimal model that has been relied on so far.

[0003] Modern DNA-technologies provide the opportunity to exploit these major genes, and this approach is a very promising route for the improvement of meat quality, especially since direct meat quality assessment is not viable for potential breeding animals. Also for other traits such as lean/fat ratio, growth rate and feed efficiency, modern DNA technology can be very effective. Also these traits are not always easy to measure in the living animal.

[0004] The evidence for several of the major genes was originally obtained using segregation analysis, i.e., without any DNA marker information. Afterwards, molecular studies were performed to detect the location of these genes on the genetic map. In practice, and except for alleles of very large effect, DNA studies are required to dissect the genetic nature of most traits of economic importance. DNA markers can be used to localise genes or alleles responsible for qualitative traits like coat color, and they can also be used to detect genes or alleles with substantial effects on

quantitative traits like growth rate, IMF<sub>2</sub> etc. In this case, the approach is referred to as QTL (quantitative trait locus) mapping, wherein a QTL comprises at least a part of the nucleic acid genome of an animal where genetic information capable of influencing said quantitative trait (in said animal or in its offspring) is located. Information at the DNA level can not only help to fix a specific major gene in a population, but also assist in the selection of a quantitative trait which is already selected for. Molecular information in addition to phenotypic data can increase the accuracy of selection and therefore the selection response.

[0005] Improving meat quality or carcass quality is not just about changing levels of traits like tenderness or marbling, but it is also about increasing uniformity. The existence of major genes provides excellent opportunities for improving meat quality because it allows large steps to be made in the desired direction. [Secondly, it]It will help to reduce variation, since we can fix relevant genes in our products. Another aspect is that selecting for major genes allows differentiation for specific markets. Studies are underway in several species, particularly, pigs, sheep, deer and beef cattle.

[0006] In particular, intense selection for meat production has resulted in animals with extreme muscularity and leanness in several livestock species. In recent years it has become feasible to map and clone several of the genes causing these phenotypes, paving the way towards more efficient [marker assisted]marker-assisted selection, targeted drug development ([performance enhancing]performance-enhancing products) and transgenesis. Mutations in the ryanodine receptor (Fuji et al, 1991; MacLennan and Phillips, 1993) and myostatin (Grobet et al, 1997; Kambadur et al, 1997; McPherron and Lee, 1997) have been shown to cause muscular hypertrophies in pigs and cattle respectively, while genes with major effects on muscularity and/or fat deposition have, for instance, been mapped to pig chromosome 4 (Andersson et al, 1994) and sheep chromosome 18 (Cocket et al, 1996).

#### DISCLOSURE OF THE INVENTION

[0007] However, although there have been successes in identifying QTLs, the information is currently of limited use within commercial breeding programmes. Many workers in this field conclude that it is necessary to identify the particular genes underlying the QTL. This is a substantial task, as the QTL region is usually relatively large and may contain many genes. Identification of the

relevant genes from the many that may be involved thus remains a significant hurdle in farm animals.

[0008] The invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising testing [said]the animal for the presence of a parentally imprinted qualitative or quantitative trait locus (QTL). Herein, a domestic animal is defined as an animal being selected or having been derived from an animal having been selected for having desired genotypic or potential phenotypic properties.

[0009] Domestic animals provide a rich resource of genetic and phenotypic variation[,]; traditionally, domestication involves selecting an animal or its offspring for having desired genotypic or potential phenotypic properties. This selection process has in the past century been facilitated by growing understanding and utilisation of the laws of Mendelian inheritance. One of the major problems in breeding programmes of domestic animals is the negative genetic correlation between reproductive capacity and production traits. This is, for example, the case in cattle (a high milk production generally results in slim cows and bulls), poultry[,] (broiler lines have a low level of egg production and layers have generally very low muscle growth), pigs (very prolific sows are, in general, fat and have comparatively less meat) or sheep (high prolific breeds have low carcass quality and vice versa). The invention now provides that knowledge of the parental imprinting character of various traits allows to select, for example, sire lines homozygous for a paternally imprinted QTL, for example, linked with muscle production or growth; the selection for such traits can thus be less stringent in dam lines in favour of the reproductive quality. The phenomenon of genetic or parental imprinting has never been utilised in selecting domestic animals[,]; it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. The invention provides a breeding programme, wherein knowledge of the parental imprinting character of a desired trait, as demonstrated herein, results in a breeding programme, for example[ in], a BLUP programme, with a modified animal model. This increases the accuracy of the breeding value estimation and speeds up selection compared to conventional breeding programmes. Until now, the effect of a parentally imprinted trait in the estimation of a conventional BLUP programme was neglected; using and understanding the parental character of the desired trait, as provided by the invention, allows selecting on parental imprinting, even without DNA testing. For example, selecting genes characterised by paternal imprinting is provided to help increase uniformity; a (terminal) parent homozygous for the

“good or wanted” alleles will pass them to all offspring, regardless of the other parent’s alleles, and the offspring will all express the desired parent’s alleles. This results in more uniform offspring. Alleles that are interesting or favourable from the maternal side [or]are often the ones that have opposite effects to alleles from the paternal side. For example, in meat animals such as pigs, alleles linked with meat quality traits such as [inta-muscular]intramuscular fat or muscle mass could be fixed in the dam lines while alleles linked with reduced [back fat]backfat could be fixed in the sire lines. Other desirable combinations are, for example, fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

[0010] In a preferred embodiment, the invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising testing a nucleic acid sample from [said]the animal for the presence of a parentally imprinted quantitative trait locus (QTL). A nucleic acid sample can, in general, be obtained from various parts of the animal’s body by methods known in the art. Traditional samples for the purpose of nucleic acid testing are blood samples or skin or mucosal surface samples, but samples from other tissues can be used as well[,]; in particular, sperm samples, oocyte or embryo samples can be used. In such a sample, the presence and/or sequence of a specific nucleic acid, be it DNA or RNA, can be determined with methods known in the art, such as hybridisation or nucleic acid amplification or sequencing techniques known in the art. The invention provides testing such a sample for the presence of nucleic acid wherein a QTL or allele associated therewith is associated with the phenomenon of parental imprinting, for example, where it is determined whether a paternal or maternal allele of said QTL is capable of being predominantly expressed in [said]the animal.

[0011] The purpose of breeding programmes in livestock is to enhance the performances of animals by improving their genetic composition. In essence, this improvement accrues by increasing the frequency of the most favourable alleles for the genes influencing the performance characteristics of interest. These genes are referred to as QTL. Until the beginning of the nineties, genetic improvement was achieved via the use of biometrical methods, but without molecular knowledge of the underlying QTL.

[0012] Since the beginning of the nineties and due to recent developments in genomics, it is conceivable to identify the QTL underlying a trait of interest. The invention now provides identifying

and using parentally imprinted QTLs which are useful for selecting animals by mapping quantitative trait loci. Again, the phenomenon of genetic or paternal imprinting has never been utilised in selecting domestic animals[,]; it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. For example, Kovacs and Kloting (Biochem. Mol. Biol. Int. 44:399-405, 1998), where parental imprinting is not mentioned, and not suggested, found linkage of a trait in female rats, but not in males, suggesting a possible sex specificity associated with a chromosomal region, which, of course, excludes parental imprinting, a phenomenon wherein the imprinted trait of one parent is preferably but genderaspecifically expressed in his or her offspring.

[0013] The invention provides the initial localisation of a parentally imprinted QTL on the genome by linkage analysis with genetic markers, and the actual identification of the parentally imprinted gene(s) and causal mutations therein. Molecular knowledge of such a parentally imprinted QTL allows for more efficient breeding designs herewith provided. Applications of molecular knowledge of parentally imprinted QTLs in breeding programmes include: [marker assisted]marker-assisted segregation analysis to identify the segregation of functionally distinct, parentally imprinted QTL alleles in the populations of interest, [marker assisted]marker-assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy[,]or selection intensity or by reducing the generation interval using the understanding of the [phenomonon]phenomenon of parental imprinting, [marker assisted]marker-assisted introgression (MAI) to efficiently transfer favourable parentally imprinted QTL alleles from a donor to a recipient population, genetic engineering of the identified parentally imprinted QTL and genetic modification of the breeding stock using transgenic technology,and development of performance enhancing products using targeted drug development exploiting molecular knowledge of said QTL.

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

--Legends to the Figures

[0014] Fig. 1: Test statistic curves obtained in QTL analyses of chromosome 2 in a Wild Boar/Large White intercross. The graph plots the F ratio testing the hypothesis of a single QTL at



a given position along the chromosome for the traits indicated. The marker map with the distances between markers in Kosambi centiMorgan is given on the X-axis. The horizontal lines represent genome-wise significant ( $P < 0.05$ ) and suggestive levels for the trait lean meat in ham; similar significance thresholds were obtained for the other traits.

[0015] Figure 2: Piétrain pig with characteristic muscular hypertrophy.

[0016] [Figure 3]Figures 3A-3C: Lodscore curves obtained in a Piétrain x Large White intercross for six phenotypes measuring muscle mass and fat deposition on pig chromosome 2. The most likely positions of the *[Igf2]*IGF2 and *MyoD* genes determined by linkage analysis with respect to the microsatellite marker map are shown.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL. [3a]3A:  $\log_{10}(H_1/H_0)$ , [3b]3B:  $\log_{10}(H_2/H_0)$ , [3c]3C:  $\log_{10}(H_3/H_0)$ .

[0017] Figure 4: A. Structure of the human *[Igf2]*IGF2 gene according to ref. 17, with aligned porcine adult liver cDNA sequence as reported in ref. 16. The position of the *nt241(G-A)* transition and *Swc9* microsatellite are shown. B. The corresponding markers were used to demonstrate the monoallelic (paternal) expression of *[Igf2]*IGF2 in skeletal muscle and liver of 10-week old fetuses. PCR amplification of the *nt421(G-A)* polymorphism and *Swc9* microsatellite from genomic DNA clearly shows the heterozygosity of the fetus, while only the paternal allele is detected in liver cDNA (*nt421(G-A)* and *Swc9*) and muscle cDNA (*Swc9*). The absence of RT-PCR product for *nt421(G-A)* [from ]in fetal muscle points towards the absence of mRNA including exon 2 in this tissue. Parental origin of the [foetal]fetal alleles was determined from the genotypes of the sire and dam (data not shown).

[0018] Figure 5: A NotI restriction map showing the relative position of BAC-PIGF2-1 (comprising INS and IGF2 genes), and BAC-PIGF2-2 (comprising IGF2 and H19 genes).

[0019] Figure 6: Nucleic acid sequences of contig 1 to contig 115 derived from BAC-PIGF2-1 which was shotgun sequenced using standard procedures and automatic sequencers.

[0020] Figure 7: Similarity between porcine contigs of figure 6 and orthologous sequences in human.

[0021] Figure 8: Nucleic acid sequences of contig 1 to contig 7 derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1) which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

[0022] Figure 9: Similarity between porcine contigs of figure 8 and orthologous sequences in human.

[0023] Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals.--

#### DETAILED DESCRIPTION OF THE INVENTION

[0024] The inventors undertook two independent experiments to determine the practical use of parental imprinting of a QTL.

[0025] In a first experiment, performed in a previously described Piétrain x Large White intercross, the likelihood of the data were computed under a model of paternal (paternal allele only expressed) and maternal imprinting (maternal allele only expressed) and compared with the likelihood of the data under a model of a conventional "Mendelian" QTL. The results strikingly demonstrated that the QTL was indeed paternally expressed, the QTL allele (Piétrain or Large White) inherited from the F<sub>1</sub> sow having no effect whatsoever on the carcass quality and quantity of the F<sub>2</sub> offspring. It was seen that very significant lodscores were obtained when testing for the presence of a paternally expressed QTL, while there was no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. The same tendency was observed for all traits, showing that the same imprinted gene is responsible for the effects observed on the different traits. Table 1 reports the maximum likelihood (ML) phenotypic means for the F<sub>2</sub> offspring sorted by inherited paternal QTL allele.

[0026] In a second experiment performed in the Wild Boar X Large White intercross, QTL analyses of body composition, fatness, meat quality, and growth traits [was]were carried out with the chromosome 2 map using a statistical model testing for the presence of an imprinting effect. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; [Table]Table 1). The clear paternal expression of a QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1).

For a given paternally imprinted QTL, implementation of [marker assisted]marker-assisted segregation analysis, selection (MAS) and introgression (MAI)[,] can be performed using genetic markers that are linked to the QTL, using genetic markers that are in linkage disequilibrium with the QTL, or using the actual causal mutations within the QTL.

[0027] Understanding the parent-of-origin effect characterising a QTL allows for its optimal use in breeding programmes. Indeed, [marker assisted]marker-assisted segregation analysis under a model of parental imprinting will yield better estimates of QTL allele effects. Moreover, it allows for the application of specific breeding schemes to optimally exploit a QTL. In one embodiment of the invention, the most favourable QTL alleles would be fixed in breeding animal lines and, for example, used to generate commercial, crossbred males by [marker assisted]marker-assisted selection (MAS, within lines) and [marker assisted]marker-assisted introgression (MAI, between lines). In another embodiment, the worst QTL alleles would be fixed in the animal lines used to generate commercial crossbred females by MAS (within lines) and MAI (between lines).

[0028] In a preferred embodiment of the invention, [said]the animal is a pig. Note, for example, that the invention provides the insight that today half of the offspring from commercially popular [Piéttrain]Piéttrain x Large White crossbred boars inherit an unfavourable Large White muscle mass QTL as provided by the invention, causing considerable loss, and the invention now, for example, provides the possibility to select the better half of the population in that respect. However, it is also possible to select commercial sow lines enriched [with the in]within the boars unfavourable alleles, allowing [to equip ]the sows to be equipped with other alleles more desirable for, for example, reproductive purposes.

[0029] In a preferred embodiment of a method provided by the invention, said QTL is located at a position corresponding to a QTL located at chromosome 2 in the pig. For example, it is known to form comparative mapping data between pig and human, including bidirectional chromosome painting[,]and that SSC2p is homologous to HSAllpter-q13<sup>11, 12</sup>. HSAllpter-q13 is known to harbour a cluster of imprinted genes: IGF2, INS2, H19, MAH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, Tapa1./CD81, Orct12, Impt1 and Ip1. The cluster of imprinted genes located in HSAllpter-q13 is characterised by 8 maternally expressed genes: H19, MASH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, TAPA1/CD81, ORCTL2, IMPT1 and IPI, and two paternally expressed genes: IGF2 and INS. However, Johanson et al. (Genomics

25:682-690, 1995) and Reik et al. (Trends in Genetics, 13:330-334, 1997) show that the whereabouts of these loci in various animals are not clear. For example, the HSA11 and MMU7 loci do not correspond among each other, the MMU7 and the SSC2 loci do not correspond, whereas the HSA11 and SSC2 loci seem to correspond, and no guidance is given where one or more of, for example, the [above identified]above-identified parentally expressed individual genes are localised on the three species' chromosomes.

**[0030]** Other domestic animals, such as cattle, sheep, poultry and fish, having similar regions in their genome harbouring such a cluster of imprinted genes or QTLs, the invention herewith provides use of these orthologous regions of other domestic animals in applying the phenomenon of parental imprinting in breeding programmes. In pigs, said cluster is mapped at around position 2p1.7 of chromosome 2[,], however, a method as provided by the invention employing (fragments of) said maternally or paternally expressed orthologous or homologous genes or QTLs [are]is advantageously used in other animals as well as for breeding and selecting purposes. For example, a method is provided wherein said QTL is related to the potential muscle mass and/or fat deposition, preferably with limited effects on other traits such as meat quality and daily gain of [said]the animal or wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) allele. Reik et al. (Trends in Genetics, 13:330-334, 1997) explain that this gene in humans is related to Beckwith-Wiedemann syndrome, an apparently parentally imprinted disease syndrome most commonly seen with human [foetuses]fetuses, where the gene has an important role in prenatal development. No relationship is shown or suggested with postnatal development relating to muscle development or fatness in (domestic) animals.

**[0031]** In a preferred embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7. In particular, the invention relates to the use of genetic markers for the telomeric end of pig chromosome 2p in marker selection (MAS) of a parentally imprinted Quantitative Trait Locus (QTL) affecting carcass yield and quality in pigs. Furthermore, the invention relates to the use of genetic markers associated with the IGF2 locus in MAS in pigs, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such

as shown in figures 4 to 10. In a preferred embodiment, the invention provides a QTL located at the distal tip of *Sus scrofa* chromosomes 2 with effects on [varies]various measurements of carcass quality and quantity, particularly muscle mass and fat deposition.

**[0032]** In a first experiment, a QTL mapping analysis was performed in a Wild Boar X Large White intercross counting 200 F<sub>2</sub> individuals. The F<sub>2</sub> animals were sacrificed at a live [eight]weight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are provided by Andersson et al.<sup>1</sup> and Andersson-Eklund et al.<sup>4</sup>

**[0033]** A QTL (without any significant effect on back-fat thickness) at an unspecified locus on the proximal end of chromosome 2 with moderate effect on muscle mass, and located about 30cM away from the parentally imprinted QTL reported here, was previously reported by the inventors; whereas the QTL as now provided has a very large effect, explaining at least 20-30% of variance, making the QTL of the present invention commercially very attractive, which is even more so because the present QTL is parentally imprinted. The marker map of chromosome 2p was improved as part of this invention by adding microsatellite markers in order to cover the entire chromosome arm. The following microsatellite markers were used: *Swc9*, *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map. Clear evidence for a QTL located at the very distal tip of 2p was obtained (Fig. 1; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population.

**[0034]** In a second experiment, QTL mapping was performed in a Piétrain X Large White intercross comprising 1125 F<sub>2</sub> offspring. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famous for their exceptional muscularity

and leanness <sup>10</sup>(Figure 2), while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring growth performance (5), muscularity (6), fat deposition (6), and meat quality (4), were recorded on all F<sub>2</sub> offspring. In order to map QTL underlying the genetic differences between these breeds, the inventors undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. The following microsatellite marker map was used to analyse chromosome 2;[.] SW2443, SWC9 and SW2623, SWR2516- (0,20) -SWR783- (0,29) -SW240- (0,20) -SW776- (0,08) -S0010- (0, 04) -SW1695-(0,36) -SWR308. Analysis of pig chromosome 2 using a Maximum Likelihood multipoint algorithm[,] revealed highly significant lodscores (up to 20) for three of the six phenotypes measuring muscularity (% lean cuts, % ham, % loin) and three of the six phenotypes measuring fat deposition (back-fat thickness (BFT), % backfat, % fat cuts) at the distal end of the short arm of chromosome 2 (Figure 1). Positive lodscores were obtained in the corresponding chromosome region for the remaining six muscularity and fatness phenotypes, however, not reaching the experiment-wise significance threshold[)] ( $\alpha=5\%$ ). There was no evidence for an effect of the corresponding QTL on growth performance (including birth weight) or recorded meat quality measurements (data not shown). To confirm this finding, the remaining sample of 355 F<sub>2</sub> offspring was genotyped for the four most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for the three genotypic means as well as the residual variance. Evidence based on [marker assisted]marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population.

[0035] These experiments, therefore, clearly indicated the existence of a QTL with a major effect on carcass quality and quantity on the telomeric end of pig chromosome arm 2p[;], the likely existence of an allelic series at this QTL with at least three alleles: Wild-Boar < Large White < Piétrain, and possibly more given the observed segregation within the Piétrain breed.

[0036] The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the *CRC* locus though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. The QTL had very

large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL, when compared to the Wild Boar allele, was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits shows that a single causative locus is involved. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output.

[0037] In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7., wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele or a [genomic]genomic area closely related thereto, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. The important role of IGF2 for prenatal development is well-documented from knockout mice as well as from its causative role in the human Beckwith-Wiedemann syndrome. This invention demonstrates an important role for the IGF2-region, also for postnatal development.

[0038] To show the role of [Igf2]IGF2, the inventors performed the following three experiments:

[0039] A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p.

[0040] A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800bp downstream of the stop

codon; a sequence comparison revealed that this microsatellite was identical to a previously described anonymous microsatellite, *Swc9*<sup>6</sup>. This marker was used in the initial QTL mapping experiments and its location on the genetic map correspond with the most likely position of the QTL both in the Piétrain X Large White and in the Large White x Wild Boar pedigree.

[0041] Analysis of skeletal muscle and liver cDNA from 10-week old [foetuses]fetuses heterozygous for a nt241 (G-A) transversion in the second exon of the porcine [IGFII]IGF2 gene and SWC9[,] shows that the [IGFII]IGF2 gene is imprinted in these tissues in the pig as well and only expressed from the paternal allele.

[0042] Based on a published porcine adult liver cDNA sequence<sup>16</sup>, the inventors designed primer pairs allowing [to amplify]amplification of the entire [*IgfII*]IGF2 coding sequence with 222 bp of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced[ indication], indicating that the coding sequences are identical in both breeds and with the published sequence. However, a G↔A transition was found in the leader sequence corresponding to exon 2 in man. Following conventional nomenclature, this polymorphism will be referred to as *nt241* (G-A). We developed a screening test for this single nucleotide polymorphism 9(SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, [*IgfII*]IGF2 was shown to colocalize with the SWC9 microsatellite marker (θ=0%), therefore virtually coinciding with the most likely position of the QTL, and well within the 95% support interval for the QTL. Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3'UTR of the [*IgfII*]IGF2 gene.

[0043] As previously mentioned, the knowledge of this QTL provides a method for the selection of animals such as pigs with improved carcass merit. Different embodiments of the invention are envisaged, including: [marker assisted]marker-assisted segregation analysis to identify the segregation of functionally distinct QTL alleles in the populations of interest; [marker assisted]marker-assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy[,]or selection intensity or by reducing the generation interval; [marker assisted]and marker-assisted introgression (MAI) to efficiently transfer favourable QTL alleles from



a donor to a recipient population, thereby enhancing genetic response in the recipient population. Implementation of embodiments [marker assisted]of marker-assisted segregation analysis, selection (MAS) and introgression (MAI)[,] can be performed using genetic markers that are linked to the QTL[;], genetic markers that are in linkage disequilibrium with the QTL, and the actual causal mutations within the QTL.

[0044] In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2p1.7., wherein said QTL is paternally expressed, i.e., is expressed from the paternal allele. In man and mouse, *[Igf]IGF2* is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9[,] shows that the same imprinting holds in the pig as well. Understanding the parent-of-origin effect characterising the QTL as provided by the invention now allows for its optimal use in breeding programmes. Indeed, today, half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele, causing considerable loss. Using a method as [provide]provided by the invention avoids this problem.

[0045] The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof comprising a parentally imprinted quantitative trait locus (QTL) or fragment thereof capable of being predominantly expressed by one parental allele. Having such a nucleic acid as provided by the invention available allows constructing transgenic animals wherein favourable genes are capable of being exclusively or predominantly expressed by one parental allele, thereby equipping the offspring of [said]the animal homozygous for a desired trait with desired properties related to that parental allele that is expressed.

[0046] In a preferred embodiment, the invention provides an isolated and/or recombinant nucleic acid or fragment derived thereof comprising a synthetic parentally imprinted quantitative trait locus (QTL) or functional fragment thereof derived from at least one chromosome. Synthetic herein describes a parentally expressed QTL wherein various elements are combined that originate from distinct locations from the genome of one or more animals. The invention provides recombinant

nucleic acid wherein sequences related to parental imprinting of one QTL are combined with sequences relating to genes or favourable alleles of a second QTL. Such a gene construct is favourably used to obtain transgenic animals wherein the second QTL has been equipped with paternal imprinting, as opposed to the inheritance pattern in the native animal from which the second QTL is derived. Such a second QTL can, for example, be derived from the same chromosome where the parental imprinting region is located, but can also be derived from a different chromosome from the same or even a different species. In the pig, such a second QTL can, for example, be related to an oestrogen receptor (ESR)-gene (Rothschild et al., PNAS, 93, 201-201, 1996) or a FAT-QTL (Andersson, Science, 263, 1771-1774, 1994) for example, derived from [an other]another pig chromosome, such as chromosome 4. A second or further QTL can also be derived from another (domestic) animal or a human.

[0047] The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof at least partly corresponding to a QTL of a pig located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7 wherein said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele, preferably at least spanning a region between INS and H19, or preferably derived from a domestic pig, such as a [Pietrain]Piétrain, Meishan, Duroc, Landrace or Large White, or from a Wild Boar. For example, a genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAG clone gave a strong, consistent signal on the terminal part of chromosome 2p. A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, *Swc9*. PGR primers were designed and the microsatellite (IGF2ms) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two among the eight Large White founders. IGF2ms was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each F<sub>2</sub> animal.

[0048] A linkage analysis using the intercross pedigree was carried out with IGF2ms and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. IGF2 was firmly assigned to 2p by highly significant [lod scores]lodscores (e.g.  $Z=89.0$ ,  $\theta=0.003$  against *Swr2516*). Multipoint analyses, including previously typed chromosome 2 markers, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443*/*Swr2516*-0.3-IGF2-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed between *Sw2443* and *Swr2516*, and the suggested proximal location of IGF2 in relation to these loci is based on a single recombinant giving a [lod score]lodscore support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

[0049] The invention furthermore provides use of a nucleic acid or functional fragment derived thereof according to [the invention in ]a method according to the invention. In a preferred embodiment, use of a method according to the invention is provided to select a breeding animal or animal destined for slaughter, or embryos or semen derived from these animals, for having desired genotypic or potential phenotypic properties. In particular, the invention provides such use wherein said properties are related to muscle mass and/or fat deposition. The QTL as provided by the invention may be exploited or used to improve, for example, lean meat content or back-fat thickness by [marker assisted]marker-assisted selection within populations or by [marker assisted]marker-assisted introgression of favourable alleles from one population to another. Examples of [marker assisted]marker-assisted selection using the QTL as provided by the invention are use of [marker assisted]marker-assisted segregation analysis with linked markers or with markers in disequilibrium to identify functionally distinct QTL alleles. Furthermore, identification of a causative mutation in the QTL is now possible, again leading to identify functionally distinct QTL alleles. Such functionally distinct QTL alleles located at the distal tip of chromosome 2p with large effects on skeletal muscle mass, the size of the heart, and on back-fat thickness are also provided by the invention. The observation of a similar QTL effect in a Large White x Wild Boar as well as in a Piétrain x Large White intercross provides proof of the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on [marker assisted]marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series as provided by the invention allows identifying causal polymorphisms

which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found, i.e., Piétrain pigs are more muscular than Large White pigs that in turn have higher lean meat content than Wild Boars. The invention furthermore provides use of the alleles as provided by the invention [for ]within line selection or for [marker assisted]marker-assisted introgression using linked markers, markers in disequilibrium or alleles comprising causative mutations.

[0050] The invention furthermore provides an animal selected by using a method according to the invention. For example, a pig characterised in being homozygous for an allele in a QTL located at a Sus scrofa chromosome 2 mapping at position 2p1.7 can now be selected and is thus provided by the invention. Since said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or said QTL comprises at least a part of a Sus scrofa insulin-like growth factor-2 (IGF2) allele, it is possible to select promising pigs to be used for breeding or to be slaughtered. In particular, an animal according to the invention which is a male is provided. Such a male, or its sperm or an embryo derived thereof, can advantageously be used in breeding animals for creating breeding lines or for finally breeding animals destined for slaughter. In a preferred embodiment of such use as provided by the invention, a male, or its sperm, deliberately selected for being homozygous for an allele causing the extreme muscular [hyperthrophy]hypertrophy and leanness, is used to produce offspring heterozygous for such an allele. Due to said allele's paternal expression, said offspring will also show the favourable traits, for example, related to muscle mass, even if the parent female has a different genetic background. Moreover, it is now possible to positively select the female(s) for having different traits, for example, related to fertility, without having a negative effect on the muscle mass trait that is inherited from the allele from the selected male. For example, earlier such males could occasionally be seen with Piétrain pigs but genetically it was not understood how to most profitably use these traits in breeding programmes.

[0051] Furthermore, the invention provides a transgenic animal, sperm and an embryo derived thereof, comprising a synthetic parentally imprinted QTL or functional fragment thereof as provided by the invention, i.e., it is provided by the invention to introduce a favourable recombinant allele; for example, to introduce the oestrogen receptor locus related to increased litter size of an

animal homozygously in a parentally imprinted region of a grandparent animal (for example, the father of a hybrid sow if the region was paternally imprinted and the grandparent was a boar); to introduce a favourable fat-related allele or muscle mass-related recombinant allele in a paternally imprinted region, and so on. Recombinant alleles that are interesting or favourable from the maternal side [or]are often the ones that have opposite effects to alleles from the paternal side. For example, in meat animals such as pigs, recombinant alleles linked with meat quality traits such as [intramuscular]intramuscular fat or muscle mass could be fixed in the dam lines while recombinant alleles linked with reduced [back fat]backfat could be fixed in the sire lines. Other desirable combinations are, for example, fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

**[0052]** The invention is further explained in the detailed description without limiting the invention.

Example 1: Wild Boar x Large White intercrosses

## Methods

**[0053]** Isolation of an IGF2 BAC clone and fluorescent *in situ* hybridisation (FISH). IGF2 primers (F:5' - GGCAAGTTCTTCCGCTAATGA-3' (SEQ. ID. NO: \_\_\_\_ ) and R:5' - GCACCGCAGAATTACGACAA-3' (SEQ. ID. NO: \_\_\_\_)) for PCR amplification of a part of the last exon and 3'UTR were designed on the basis of a porcine IGF2 cDNA sequence (GenBank X56094). The primers were used to screen a porcine BAC library and the clone 253G10 was isolated. Crude BAC DNA was prepared as described<sup>24</sup>. The BAC DNA was linearized with *EcoRV* and purified with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two days at room temperature and then kept at -20°C until use. FISH analysis was carried out as previously described<sup>25</sup>. The final concentration of the probe in the hybridisation mix was 10 ng/μl. Repetitive sequences were suppressed with standard concentrations

of porcine genomic DNA. After post-hybridisation washing, the biotinylated probe was detected with two layers of avidin-FITC (Vector A-2011). The chromosomes were counterstained with 0.3 mg/ml DAPI (4, 6-Diamino-2-phenylindole; Sigma D9542), which produced a G-banding like pattern. No posthybridisation banding was needed, since chromosome 2 is easily recognized without banding. A total of 20 metaphase spreads were examined under an Olympus BX-60 fluorescence microscope connected to an IMAC-CCD S30 video camera and equipped with an ISIS 1.65 (Metasystems) software.

Sequence, microsatellite, and linkage analysis.

**[0054]** About two  $\mu\text{g}$  of linearized and purified BAC DNA was used for direct sequencing with 20 pmoles of primers and BigDye Terminator chemistry (Perkin Elmer, USA). DNA sequencing was done from the 3' end of the last exon towards the 3' end of the UTR until a microsatellite was detected. A primer set (F:5'-GTTTCTCCTGTACCCACACGCATCCC-3' (SEQ. ID. NO: \_\_\_\_ ) and R: 5' -Fluorescein- CTACAAGCTGGGCTCAGGG-3') (SEQ. ID. NO: \_\_\_\_ ) was designed for the amplification of the IGF2 microsatellite which is about 250 bp long and located approximately 800 bp downstream from the stop codon. The microsatellite was PCR amplified using fluorescently labeled primers and the genotyping was carried out using an ABI377 sequencer and the GeneScan/Genotyper softwares (Perkin Elmer, USA). Two-point and multipoint linkage [analysis]analyses were done with the Cri-Map software<sup>26</sup>.

Animals and phenotypic data.

**[0055]** The intercross pedigree comprised two European Wild Boar males and eight Large White females, 4 F<sub>1</sub> males and 22 F<sub>1</sub> females, and 200 F<sub>2</sub> progeny<sup>1</sup>. The F<sub>2</sub> animals were sacrificed at a live weight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits [are]is provided by Andersson *et al.*<sup>1</sup> and Andersson-Eklund *et al.*<sup>4</sup>

Statistical analysis.

[0056] Interval mapping for the presence of QTL [were]was carried out [with]using a least squares method developed for the analysis of crosses between outbred lines<sup>27</sup>. The method is based on the assumption that the two divergent lines are fixed for alternative QTL alleles. There are four possible genotypes in the F<sub>2</sub> generation [as]was regards to the grandparental origin of the alleles at each locus. This makes it possible to fit three effects: additive, dominance, and imprinting<sup>2</sup>. The latter is estimated as the difference between the two types of heterozygotes, the one receiving the Wild Boar allele through an F<sub>1</sub> sire and the one receiving it from an F<sub>1</sub> dam. An F-ratio was calculated using this model (with 3 d.f.) versus a reduced model without a QTL effect for each cM of chromosome 2. The most likely position of a QTL was obtained as the location giving the highest F-ratio. Genome-wise [significance]significant thresholds were obtained empirically by a permutation test<sup>28</sup> as described<sup>2</sup>. The QTL model including an imprinting effect was compared with a model without imprinting (with 1 d.f.) to test whether the imprinting effect was significant.

[0057] The statistical models also included the fixed effects and [covariates]covariants that were relevant for the respective traits; see Andersson-Eklund *et al.*<sup>4</sup> for a more detailed description of the statistical models used. Family was included to account for background genetic effects and maternal effects. Carcass weight was included as a [covariate]covariant to discern QTL effects on correlated traits, which means that all results concerning body composition were compared at equal weights. Least-squares means for each genotype class at the IGF2 locus were estimated with a single point analysis using Procedure GLM of SAS<sup>29</sup>; the model included the same fixed effects and [covariates]covariants as used in the interval mapping analyses. The QTL shows a clear parent of origin-specific expression and the map position coincides with that of the insulin-like growth factor II gene (IGF2), indicating IGF2 as the causative gene. A highly significant segregation distortion (excess of Wild Boar-derived alleles) was also observed at this locus. The results demonstrate an important effect of the IGF2 region on postnatal development and it is possible that the presence of a paternally expressed IGF2-linked QTL in humans and in rodent model organisms has so far been

overlooked due to experimental design or statistical treatment of data. The study has also important implications for quantitative genetics theory and practical pig breeding.

**[0058]** IGF2 was identified as a positional candidate gene for this QTL due to the observed similarity between pig chromosome 2p and human chromosome 11p. A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong, consistent signal on the terminal part of chromosome 2p (Fig. 1). A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, *Swc9*<sup>6</sup>. PCR primers were designed and the microsatellite (IGF2ms) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two among the eight Large White founders. IGF2ms was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each F<sub>2</sub> animal.

**[0059]** A linkage analysis using the intercross pedigree was carried out with IGF2ms and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. IGF2 was firmly assigned to 2p by highly significant [lod scores] lodscores (e.g.,  $Z=89.0$ ,  $\theta=0.003$  against *Swr2516*). Multipoint analyses, including previously typed chromosome 2 markers<sup>8</sup>, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443*/*Swr2516*-0.3-IGF2-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed between *Sw2443* and *Swr2516*, and the suggested proximal location of IGF2 in relation to these loci is based on a single recombinant giving a [lod score] lodscore support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

**[0060]** QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map using a statistical model testing for the possible presence of an imprinting effect as expected for IGF2. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub>



population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits strongly suggests a single causative locus. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output. The clear paternal expression of this QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). It is worth noticing though that there was a non-significant trend towards less extreme values for the two heterozygous classes, in particular for the estimated effect on the area of longissimus dorsi. This may be due to chance, but could have a biological explanation, e.g., that there is some expression of the maternally inherited allele or that there is a linked, non-imprinted QTL with minor effects on the traits in question.

**[0061]** The IGF2-linked QTL and the *FAT1* QTL on chromosome 4 1, 9 are by far the two loci with the largest effect on body composition and fatness segregating in this Wild Boar intercross. The IGF2 QTL controls primarily muscle mass whereas *FAT1* has major effects on fat deposition including abdominal fat, a trait that was not affected by the IGF2 QTL (Fig. 2). No significant interaction between the two loci was indicated and they control a very large proportion of the residual phenotypic variance in the  $F_2$  generation. A model including both QTLs explains 33.1% of the variance for percentage of lean meat in ham, 31.3% for the percentage of lean meat plus bone in back, and 26.2% for average [back fat]backfat depth (compare with a model including only chromosome 2 effects, Table 1). The two QTLs must have played a major role in the response during selection for lean growth and muscle mass in the Large White domestic pig.

**[0062]** A highly significant segregation distortion was observed in the IGF2 region (excess of Wild Boar-derived alleles) as shown in Table 1 ( $\chi^2=11.7$ , d.f.=2;  $P=0.003$ ). The frequency of Wild Boar-derived IGF2 alleles was 59% in contrast to the expected 50% and there was twice as many “Wild Boar” as “Large White” homozygotes. This deviation was observed with all three loci at the

distal tip and is thus not due to typing errors. The effect was also observed with other loci but the degree of distortion decreased as a function of the distance to the distal tip of the chromosome. Blood samples for DNA preparation were collected at 12 weeks of age and we are convinced that the deviation from expected Mendelian ratios was present at birth as the number of animals lost prior to blood sampling was not sufficient enough to cause a deviation of this magnitude. No other of the more than 250 loci analysed in this pedigree show such a marked segregation distortion (L. Andersson, unpublished). The segregation distortion did not show an imprinting effect, as the frequencies of the two reciprocal types of heterozygotes were identical (Table 1). This does not exclude the possibility that the QTL effects and the segregation distortion are controlled by the same locus. The segregation distortion [maybe] may be due to meiotic drive favouring the paternally expressed allele during gametogenesis, as the F<sub>1</sub> parents were all sired by Wild Boar males. Another possibility is that the segregation distortion may be due to codominant expression of the maternal and paternal allele in some tissues and/or during a critical period of embryo development. Biallelic IGF2 expression has been reported to occur to some extent during human development<sup>10, 11</sup> and, interestingly, a strong influence of the parental species background on IGF2 expression was recently found in a cross between *Mus musculus* and *Mus spretus*<sup>12</sup>. It is also interesting that a VNTR polymorphism at the insulin gene, which is very closely linked to IGF2, is associated with size at birth in humans<sup>13</sup>. It is possible that the IGF2-linked QTL in pigs has a minor effect on birth weight, but in our data it was far from significant (Fig. 2) and there was no indication of an imprinting effect.

[0063] This study is an advance in the general knowledge concerning the biological importance of the IGF2 locus. The important role of IGF2 for prenatal development is well-documented from knock-out mice<sup>14</sup> as well as from its causative role in the human Beckwith-Wiedemann syndrome<sup>15</sup>. This study demonstrates an important role for the IGF2-region also for postnatal development. It should be stressed that our intercross between outbred populations is particularly powerful to detect QTL with a parent of origin-specific effect on a multifactorial trait. This is because multiple alleles (or haplotypes) are segregating and we could deduce whether a heterozygous F<sub>2</sub> animal received the Wild Boar allele from the F<sub>1</sub> male or female. It is quite possible that the segregation of a paternally expressed IGF2-linked QTL affecting a trait like obesity has been overlooked in human studies or in intercrosses between inbred rodent populations because of

experimental design or statistical treatment of data. An imprinting effect cannot be detected in an intercross between two inbred lines as only two alleles are segregating at each locus. Our result has, therefore, significant bearings on the future analysis of the association between genetic polymorphism in the Insulin-IGF2 region and Type I diabetes<sup>16</sup>, obesity<sup>17</sup>, and variation in birth weight<sup>13</sup> in humans, as well as for the genetic dissection of complex traits using inbred rodent models. A major impetus for generating an intercross between the domestic pig and its wild ancestor was to explore the possibilities to map and identify major loci that have responded to selection. We have now showed that two single QTLs on chromosome 2 (this study) and 4<sup>1, 2</sup> explain as much as one third of the phenotypic variance for lean meat content in the F<sub>2</sub> generation. This is a gross deviation from the underlying assumption in the classical infinitesimal model in quantitative genetics theory, namely, that quantitative traits are controlled by an infinite number of loci, each with an infinitesimal effect. If a large proportion of the genetic difference between two divergent populations (e.g., Wild Boar and Large White) is controlled by a few loci, one would assume that selection would quickly fix QTL alleles with large effects leading to a selection plateau. However, this is not the experience in animal breeding programmes or selection experiments where good, persistent, long-term selection responses are generally obtained, provided that the effective population size is reasonably large<sup>18</sup>. A possible explanation for this paradox is that QTL alleles controlling a large proportion of genetic differences between two populations may be due to several consecutive mutations; this may be mutations in the same gene or at several closely linked genes affecting the same trait. It has been argued that new mutations contribute substantially to long-term selection responses<sup>19</sup>, but the genomic distribution of such mutations [are]is unknown.

**[0064]** The search for a single causative mutation is the paradigm [as regards]regarding the analysis of genetic defects in mice and monogenic disorders in humans. We propose that this may not be the case for loci that have been under selection for a large number of generations in domestic animals, crops, or natural populations. This hypothesis predicts the presence of multiple alleles at major QTL. It gains some support from our recent characterisation of porcine coat color variation. We have found that both the alleles for dominant white color and for black-spotting differ from the corresponding wild-type alleles by at least two consecutive mutations with phenotypic effects at the *KIT* and *MC1R* loci, respectively<sup>20, 21</sup>. In this context, it is highly interesting that in the accompanying

example we have identified a third allele at the IGF2-linked QTL. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found, i.e., Piétrain pigs are more muscular than Large White pigs [that], which in turn have a higher lean meat content than Wild Boars.

[0065] There are good reasons to decide that IGF2 is the causative gene for the now reported QTL. Firstly, there is a perfect agreement in map localization (Fig. 2). Secondly, it has been shown that IGF2 is paternally expressed in mice, humans, and now in pigs, like the QTL. There are several other imprinted genes in the near vicinity of IGF2 in mice and humans (*Mash2*, *INS2*, *H19*, *KVLQT1*, *TAPAI/CD81*, and *CDKN1C/p57<sup>KIP2</sup>*) but only IGF2 is paternally expressed in adult tissues<sup>22</sup>. We believe that this locus provides a unique opportunity for molecular characterisation of a QTL. The clear paternal expression can be used to exclude genes that do not show this mode of inheritance. Moreover, the presence of an allelic series should facilitate the difficult distinction between causative mutations and linked neutral polymorphism. We have already shown that there is no difference in coding sequence between IGF2 alleles from Piétrain and Large White pigs suggesting that the causative mutations occur in regulatory sequences. An obvious step is to sequence the entire IGF2 gene and its multiple promoters from the three populations. The recent report that a VNTR polymorphism in the promoter region of the insulin (*INS*) gene affects IGF2 expression<sup>23</sup> suggests that the causative mutations may be at a considerable distance from the IGF2 coding sequence.

[0066] The results have several important implications for the pig breeding industry. They show that genetic imprinting is not an esoteric academic question but [need]needs to be considered in practical breeding programmes. The detection of three different alleles in Wild Boar, Large White, and Piétrain populations indicates that further alleles at the IGF2-linked QTL segregate within commercial populations. The paternal expression of the QTL facilitates its detection using large paternal half-sib families as the female contribution can be ignored. The QTL is exploited to improve lean meat content by [marker assisted]marker-assisted selection within populations or by [marker assisted]marker-assisted introgression of favourable alleles from one population to another.

## Example 2: Piétrain x Large White intercrosses

### Methods

[0067] *Pedigree material*: The pedigree material utilised to map QTL was selected from a previously described Piétrain x Large White F2 pedigree comprising > 1,800 individuals<sup>6, 7</sup>. To assemble this F2 material, 27 Piétrain boars were mated to 20 Large White sows to generate an F1 generation comprising 456 individuals. 31 F1 boars were mated to unrelated 82 F1 sows from 1984 to 1989, yielding a total of 1862 F2 offspring. F1 boars were mated on average to 7 females, and F1 sows to an average of 2, 7 males. Average offspring per boar [were]was 60 and per sow was 23.

[0068] *Phenotypic information*: (i) *Data collection*: A total of 21 distinct phenotypes were recorded in the F2 generation<sup>6, 7</sup>. These included:

- five growth traits: birth weight (g), weaning weight (Kg), grower weight (Kg), finisher weight (Kg) and average daily gain (ADG; Kg/day; grower to [finisher]finisher period);
- two body proportion measurements: carcass length (cm); and a conformation score (0 to 10 scale; ref.6);
- ten measurements of carcass composition obtained by dissection of the chilled carcasses 24 hours after slaughter. These include measurements of muscularity: % ham (weight hams/carcass weight), % loin (weight loin/carcass weight), % shoulder (weight shoulder/carcass weight), % lean cuts (% ham + % loin + % shoulder); and measurements of fatness: average back-fat thickness (BFT; cm), % backfat (weight backfat/carcass weight), % belly (weight belly/carcass weight), % leaf fat (weight leaf fat/carcass weight), % jowl (weight jowl/carcass weight), and “% fat cuts” (% backfat + % belly + % [leaf]leaf fat + % jowl).
- four meat quality measurements: pH<sub>LD1</sub> (*Longissimus dorsi* 1 hour after slaughter), pH<sub>LD24</sub> (*Longissimus dorsi* 24 hours after slaughter), pH<sub>GL</sub> (*Gracilis* 1 hour after slaughter) and pH<sub>GL24</sub> (*Gracilis* 24 hours after slaughter). (ii) *Data processing*: Individual phenotypes were preadjusted for fixed effects (sire, dam, CRC genotype, sex, year-season, parity) and [covariates]covariates (litter size, birth weight, weaning weight, grower weight, finisher weight) that proved to significantly affect the corresponding trait. Variables included in the model were selected by stepwise regression.

[0069] *Marker genotyping:* Primer pairs utilised for PCR amplification of microsatellite markers are as described<sup>19</sup>. Marker genotyping was performed as previously described<sup>20</sup>. Genotypes at the *CRC* and *MyoD* loci were determined using conventional methods as described<sup>1,12</sup>. The LAR test for the [Igf2]IGF2 SNP was developed according to Baron et al.<sup>21</sup> using a primer pair for PCR amplification (5'-CCCCTGAACTTGAGGACGAGCAGCC-3' (SEQ. ID. NO: \_\_\_\_); 5'-ATCGCTGTGGGCTGGGTGGGCTGCC-3') (SEQ. ID. NO: \_\_\_\_ and a set of three primers for the LAR step (5'-FAM- CGCCCCAGCTGCCCCCAG-3' (SEQ. ID. NO: \_\_\_\_); 5' -HEX- CGCCCCAGCTGCCCCCAA-3' (SEQ. ID. NO: \_\_\_\_); 5' -CCTGAGCTGCAGCAGGCCAG-3') (SEQ. ID. NO: \_\_\_\_).

[0070] *Map construction:* Marker maps were constructed using the TWOPOINT, BUILD and CHROMPIC options of the CRIMAP package<sup>22</sup>. To allow utilisation of this package, full-sib families related via the boar or sow were disconnected and treated independently. By doing so, some potentially usable information was neglected, yielding, however, unbiased estimates of recombination rates.

[0071] *QTL mapping: (i) Mapping Mendelian QTL:* Conventional QTL mapping was performed using a multipoint maximum likelihood method. The applied model assumed one segregating QTL per chromosome[,] and fixation of alternate QTL alleles in the respective parental lines, Piétrain (P) and Large White (LW). A specific analysis programme had to be developed to account for the missing genotypes of the parental generation, resulting in the fact that the parental origin of the F1 chromosomes could not be determined. Using a typical “interval mapping” strategy, [an]a hypothetical QTL was moved along the marker map using user-defined steps. At each position, the likelihood ( $L$ ) of the pedigree data was computed as:

$$L = \sum_{\varphi=1}^{2^r} \prod_{i=1}^n \sum_{G=1}^4 (P(G|M_p, \theta, \varphi) P(y_i|G))$$

$P$  or right [chromosome]chromosome  $P$ ), there is a total of  $2^r$  combinations for  $r$  F1 parents.

$$\prod_{i=1}^n n \text{ F2}$$

$$\sum_{G=1}^4 i\text{th F2 offspring, over the four possible QTL genotypes:}$$

*P/P, P/LW, LW/P and LW/LW*

$P(G|M_i, \theta, \phi)M_i$ : the marker genotype of the  $i$ th F2 offspring and its F1 parents, (ii)  $\phi$ : the vector of recombination rates between adjacent markers and between the hypothetical QTL and its flanking markers, and (iii)  $\theta$  the considered marker-QTL phase combination of the F1 parents.

Recombination rates and marker linkage phase of F1 parents are assumed to be known when computing this probability. Both were determined using CRIMAP in the map construction phase (see above).

$P(y_i|G)y_i$  of offspring  $i$ , given the QTL genotype under consideration. This probability is computed from the normal density function:

$$P(y_i|G) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(y_i - \mu_G)^2}{2\sigma^2}}$$

$\mu_G$  is the phenotypic mean of the considered QTL genotype (PP, PL, LP or LL) and  $\sigma^2$  the residual variance.  $\sigma^2$  was considered to be the same for the four QTL genotypic classes. The values of  $\mu_{PP}$ ,  $\mu_{PL} = \mu_{LP}$ ,  $\mu_{LL}$  and  $\sigma^2$  maximizing  $L$  were determined using the GEMINI optimisation routine<sup>23</sup>. The likelihood obtained under this alternative  $H_1$  hypothesis was compared with the likelihood obtained under the null hypothesis  $H_0$  of no QTL, in which the phenotypic means of the four QTL genotypic classes were forced to be identical. The difference between the logarithms of the corresponding

likelihoods yields a lodscore measuring the evidence in favour of a QTL at the corresponding map position.

(ii) *Significance thresholds:* Following Lander & Botstein<sup>24</sup>, lodscore thresholds ( $T$ ) associated with a chosen genome-wise significance level[, ] were computed such that:

$$\alpha = (C+9.21GT)\chi^2_2(4.6T)$$

$C$  corresponds to the number of chromosomes (= 19),  $G$  corresponds to the length of the genome in Morgans (= 29), and  $\chi^2_2(4.6T)$  denotes one minus the cumulative distribution function of the chi-squared distribution with 2 d.f. Single point 21n(LR) were assumed to be distributed as a chi-squared distribution with two degrees of freedom, as we were fitting both an additive and dominance component. To account for the fact that we were analysing multiple traits, significance levels were adjusted by applying a Bonferoni correction corresponding to the effective number of independent traits that were analysed. This effective number was estimated at 16 following the approach described by Spelman et al.<sup>25</sup>. Altogether, this allowed us to set the lodscore threshold associated with an experiment—wise significance level of 5% at 5.8. When attempting to confirm the identified QTL in an independent sample, the same approach was used, however, setting  $C$  at 1,  $G$  at 25cM and correcting for the analysis of 4.5 independent traits (as only six traits were analysed in this sample). This yielded a lodscore threshold associated with a Type I error of 5% of 2.

(iii)[.] *Testing for an imprinted QTL:* To test for an imprinted QTL, we assumed that only the QTL alleles transmitted by the parent of a given sex would have an effect on phenotype, the QTL alleles transmitted by the other parent being “neutral”. The likelihood of the pedigree data under this hypothesis was computed using equation 1. To compute  $P(y_i | G)$ , however, the phenotypic means of the four QTL genotypes were set at  $\mu_{PP} = \mu_{PL} = \mu_P$  and  $\mu_{LP} = \mu_{LL} = \mu_L$  to test for a QTL for which the paternal allele only is expressed, and  $\mu_{PP} = \mu_{LP} = \mu_P$  and  $\mu_{PL} = \mu_{LL} = \mu_L$  to test for a QTL for which the maternal allele only is expressed. It is assumed in this notation that the first subscript refers to the paternal allele, the second subscript to the maternal allele.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  testing the presence of a Mendelian QTL[;],  $H_2$  testing the presence of a paternally expressed QTL, and  $H_3$  testing the presence of a maternally expressed QTL.



[0072] *RT-PCR*: Total RNA was extracted from skeletal muscle according to Chirgwin et al.<sup>26</sup>[.] RT-PCR was performed using the Gene-Amp RNA PCR Kit (Perkin-Elmer) The PCR products were purified using QiaQuick PCR Purification kit (Qiagen) and sequenced using Dye terminator Cycle Sequencing Ready Reaction (Perkin Elmer) and an ABI373 automatic sequencer. In example 2 we report the identification of a QTL with major effect on muscle mass and fat deposition mapping to porcine 2p1.7. The QTL shows clear evidence for parental imprinting strongly suggesting the involvement of the *[Igf]/IGF2* locus.

[0073] A Piétrain X Large White intercross comprising 1125 F<sub>2</sub> offspring was generated as described<sup>6, 7</sup>. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrain are famed for their exceptional muscularity and *[leannes<sup>8</sup>]/leanness<sup>8</sup>* (Figure 2), while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring (i) growth performance (5), (ii) muscularity (6), (iii) fat deposition (6), and (iv) meat quality (4)[.] were recorded on all F<sub>2</sub> offspring.

[0074] In order to map QTL underlying the genetic differences between these breeds, we undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. Analysis of pig chromosome 2 using a ML multipoint algorithm[,] revealed highly significant lodscores (up to 20) for six of the 12 phenotypes measuring muscularity and fat deposition at the distal end of the short arm of chromosome 2 (Figure 3a). Positive lodscores were obtained for the remaining six phenotypes, however, not reaching the genome-wise significance threshold (= 5%). To confirm this finding, the remaining sample of 355 F<sub>2</sub> offspring was genotyped for the five most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for the three genotypic means as well as the corresponding residual variance.

[0075] Bidirectional chromosome painting establishes a correspondence between SSC2p and HSA1pter-q13<sup>9, 10</sup>. At least two serious candidate genes map to this region in man: the myogenic basic helix-loop-helix factor, *MyoD*, maps to HSA11p15.4, while *[Igf]/IGF2* maps to HSA11p15.5. *MyoD* is a [well known]well-known key regulator of myogenesis and is one of the first myogenic markers to be switched on during development<sup>11</sup>. A previously described amplified sequence

polymorphism in the porcine *MyoD* gene<sup>12</sup> proved to segregate in our F<sub>2</sub> material, which was entirely genotyped for this marker. Linkage analysis positioned the *MyoD* gene in the SW240-SW776 (odds > 1000) interval, therefore, well outside the lod-2 drop off support interval for the QTL (figure 1). *[Igf]/IGF2* is known to enhance both proliferation and differentiation of myoblasts *in vitro*<sup>13</sup> and to cause a muscular hypertrophy when overexpressed *in vivo*. Based on a published porcine adult liver cDNA sequence<sup>14</sup>, we designed primer pairs allowing us to amplify the entire *[Igf2]/IGF2* coding sequence with 222 bp of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced, indicating that the coding sequences [was]were identical in both breeds and with the published sequence. However, a G A transition was found in the leader sequence corresponding to exon 2 in man (Figure 4). We developed a screening test for this single nucleotide polymorphism (SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, *[Igf]/IGF2* was shown to colocalize with the SWC9 microsatellite marker (= 0%), therefore, located at approximately 2 centimorgan from the most likely position of the QTL and well within the 95% support interval for the QTL (figure 1). Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3' UTR of the *[Igf]/IGF2* gene. Combined with available comparative mapping data for the PGA and FSH loci, these results suggest the occurrence of an interstitial inversion of a chromosome segment containing *MyoD*, but not *[igf]/IGF2* which has remained telomeric in both species.

[0076] *[Igf2]/IGF2*, therefore, appeared as a strong positional allele having the observed QTL effect. In man and mouse, *[Igf]/IGF2* is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues<sup>15</sup>. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9[,] shows that the same imprinting holds in this tissue in the pig as well (Figure 4). Therefore, if *[Igf]/IGF2* were responsible for the observed effect, and knowing that only the paternal *[Igf2]/IGF2* allele is expressed, one can predict that (i) the paternal allele transmitted by Fl boars (P or LW) would have an effect on the phenotype of F2 offspring, (ii) the maternal allele transmitted by Fl sows (P or LW) would have no effect on phenotype of F2 offspring, and (iii) the likelihood of the data would be superior under a model of a bimodal (1:1) F2 population sorted by inherited paternal allele when compared to a conventional "Mendelian" model of a trimodal (1:2:1)

F2 population. The QTL mapping programmes were adapted in order to allow testing of the corresponding hypotheses.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL.

[0077] Figure 3 summarizes the obtained results. [Figure]Figures 3a, 3b and 3c respectively show the lodscore curves corresponding to  $\log_{10} (H_2/H_0)$ ,  $\log_{10} (H_3/H_0)$  and  $\log_{10} (H_2/H_1)$ . It can be seen that very significant lodscores are obtained when testing for the presence of a paternally expressed QTL, while there is no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. Also, the hypothesis of a paternally expressed QTL is significantly more likely ( $\log_{10} (H_2/H_1) > 3$ ) than the hypothesis of a "Mendelian" QTL for all examined traits. The fact that the same tendency is observed for all traits indicates that it is likely the same imprinted gene that is responsible for the effects observed on the different traits. Table 2 reports the ML phenotypic means for the F2 offspring sorted by inherited paternal QTL allele. Note that when performing the analysis under a model of a mendelian QTL, the Piétrain and Large White QTL alleles appeared to behave in an additive fashion, the heterozygous genotype exhibiting a phenotypic mean corresponding exactly to the midpoint between the two [homzygous]homozygous genotypes. This is exactly what one would predict when dealing with an imprinted QTL as [halve]half of the heterozygous offspring are expected to have inherited the P allele from their sire, the other half the LW allele.

[0078] These data, therefore, confirmed our hypothesis of the involvement of an imprinted gene expressed exclusively from the paternal allele. The fact that the identified chromosomal segment coincides precisely with an imprinted domain documented in man and mice strongly implicates the orthologous region in pigs. At least seven imprinted genes mapping to this domain have been documented ([Igf2]IGF2, Ins2, H19, Mash2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1 and TDAG51) (ref. 15 and Andrew Feinberg, personal communication). Amongst these, only [Igf2]IGF2 and Ins2 are paternally expressed. While we cannot exclude that the observed QTL effect is due to an as of yet unidentified imprinted gene in this region, its reported effects on myogenesis *in vitro* and *in vivo*<sup>13</sup> strongly implicate [Igf2]IGF2. Particularly the muscular hypertrophy observed in transgenic mice overexpressing [Igf2]IGF2 from a [muscle specific]muscle-specific promotor are in support of this

hypothesis (Nadia Rosenthal, personal communication). Note that allelic variants of the *INS* VNTR have recently been shown to be associated with size at birth in man<sup>16</sup>, and that the same VNTR has been shown to affect the level of [*Igf2*]*IGF2* expression<sup>17</sup>.

[0079] The observation of the same QTL effect in a Large White x Wild Boar intercross indicates the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on [marker assisted]marker-assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series might be invaluable in identifying the causal polymorphisms which - based on the [quantitative]quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations.

[0080] The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the *CRC* locus<sup>6,7</sup> though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. Understanding the parent-of-origin effect characterising this locus will allow for its optimal use in breeding programmes. Indeed, today, half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing considerable loss.

[0081] The QTL described in this work is the second example of a gene affecting muscle development in livestock species that exhibits a non-mendelian inheritance pattern. Indeed, we have previously shown that the callipyge locus (related to the qualitative trait wherein muscles are doubled) is characterised by polar overdominance in which only the heterozygous individuals that inherit the CLPG mutation from their sire express the double-muscling phenotype<sup>5</sup>. This demonstrates that parent-of-origin effects affecting genes underlying production traits in livestock might be relatively common.

Example 3:

Generating a reference sequence of *IGF2* and flanking loci in the pig.

[0082] The invention provides an imprinted QTL with a major effect on muscle mass mapping to the IGF2 locus in the pig, and use of the QTL as a tool in [marker assisted]marker-assisted selection. To fine tune this tool for [marker assisted]marker-assisted selection, as well as to further identify a causal mutation, we have further generated a reference sequence encompassing the entire porcine IGF2 sequence as well as that from flanking genes.

[0083] To achieve this, we screened a porcine BAC library with IGF2 probes and identified two BACs. BAC-PIGF2-1 proved to contain the INS and IGF2 genes, while BAC-PIGF2-2 proved to contain the IGF2 and H19 genes. The NotI map as well as the relative position of the two BACs is shown in Figure 5. BAC-PIGF2-1 was shotgun sequenced using standard procedures and automatic sequencers. The resulting sequences were assembled using standard software yielding a total of 115 contigs. The corresponding sequences are reported in figure 6. Similarity searches were performed between the porcine contigs and the orthologous sequences in [human]humans. Significant homologies were detected for 18 contigs and are reported in Figure 7.

[0084] For BAC-PIGF2-2, the 24 Kb NotI fragment not present in BACPIGF2-1 was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers. Resulting sequences were assembled using the Phred-Phrap-Consed programme suit, yielding seven distinct contigs (figure 8). The contig sequences were aligned with the corresponding orthologous human sequences using the compare and dotplot programmes of the GCG suite. Figure 9 [syrnmarizes]summarizes the corresponding results.

Example 4: Identification of DNA sequence polymorphisms in the IGF2 and flanking loci.

[0085] Based on the reference sequence obtained as described in Example 1, we resequenced part of the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals, allowing identification of DNA sequence polymorphisms such as reported in figure 10.

[Legends to the Figures

Fig. 1: Test statistic curves obtained in QTL analyses of chromosome 2 in a Wild Boar/Large White intercross. The graph plots the F ratio testing the hypothesis of a single QTL at a given position along the chromosome for the traits indicated. The marker map with the distances between markers in Kosambi centiMorgan is given on the X-axis. The horizontal lines represent genome-wise significant ( $P < 0.05$ ) and suggestive levels for the trait lean meat in ham; similar significance thresholds were obtained for the other traits.

Figure 2: Piétrain pig with characteristic muscular hypertrophy.

[Figure 3] Figures 3A-3C: Lodscore curves obtained in a Piétrain x Large White intercross for six phenotypes measuring muscle mass and fat deposition on pig chromosome 2. The most likely positions of the [*Igf2*]IGF2 and *MyoD* genes determined by linkage analysis with respect to the microsatellite marker map are shown.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL. [3a]3A:  $\log_{10}(H_1/H_0)$ , [3b]3B:  $\log_{10}(H_2/H_0)$ , [3c]3C:  $\log_{10}(H_3/H_0)$ .

Figure 4: A. Structure of the human [*Igf2*]IGF2 gene according to ref. 17, with aligned porcine adult liver cDNA sequence as reported in ref. 16. The position of the *nt241*(*G-A*) transition and *Swc9* microsatellite are shown. B. The corresponding markers were used to demonstrate the monoallelic (paternal) expression of [*Igf2*]IGF2 in skeletal muscle and liver of 10-week old fetuses. PCR amplification of the *nt421* (*G-A*) polymorphism and *Swc9* microsatellite from genomic DNA clearly shows the heterozygosity of the fetus, while only the paternal allele is detected in liver cDNA (*nt421*(*G-A*) and *Swc9*) and muscle cDNA (*Swc9*). The absence of RT-PCR product for *nt421*(*G-A*) [from ]in fetal muscle points towards the absence of mRNA including exon 2 in this tissue. Parental origin of the [foetal]fetal alleles was determined from the genotypes of the sire and dam (data not shown).

Figure 5: A NotI restriction map showing the relative position of BAC-PIGF2-1 (comprising INS and IGF2 genes), and BAC-PIGF2-2 (comprising IGF2 and H19 genes).

Figure 6: Nucleic acid sequences of contig 1 to contig 115 derived from BAC-PIGF2-1 which was shotgun sequenced using standard procedures and automatic sequencers.

Figure 7: Similarity between porcine contigs of figure 6 and orthologous sequences in human.

Figure 8: Nucleic acid sequences of contig 1 to contig 7 derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1) which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

Figure 9: Similarity between porcine contigs of figure 8 and orthologous sequences in human.

Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals.]

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Table 1 Summary of QTL analysis for pig chromosome 2 in a Wild Boar/Large White intercross<sup>1</sup>

Trait	F ratio <sup>2</sup> <i>QTL</i>	<i>Imprinting</i>	Map position <sup>3</sup>	Percent of F <sub>2</sub> variance <sup>4</sup>	Least squares means <sup>5</sup>		
					<i>W<sup>P</sup>/W<sup>M</sup></i>	<i>W<sup>P</sup>/L<sup>M</sup></i>	<i>L<sup>P</sup>/W<sup>M</sup></i>
<i>L<sup>P</sup>/L<sup>M</sup></i>							
<u>Body composition traits</u>							
Lean meat in ham, %	24.4***	19.1***	0	30.6	n=62 63.6 <sup>a</sup>	n=43 64.2 <sup>a</sup>	n=30 67.3 <sup>b</sup>
Lean meat mass in ham, kg	18.1***	16.8***	1	24.3	4.69 <sup>a</sup>	4.72 <sup>a</sup>	5.02 <sup>b</sup>
Lean meat + bone in back, %	12.2**	9.6**	0	17.4	66.3 <sup>a</sup>	66.7 <sup>a</sup>	70.8 <sup>b</sup>
Longissimus muscle area, cm <sup>2</sup>	10.3**	4.8*	1	15.4	31.9 <sup>a</sup>	33.0 <sup>a</sup>	35.2 <sup>b</sup>
<u>Fatness traits</u>							
Average [back fat backfat depth, mm	7.1*	8.7**	0	10.4	27.2 <sup>a</sup>	27.7 <sup>a</sup>	24.7 <sup>b</sup>
<u>Weight of internal organs</u>							
Heart, gram	9.7**	11.4***	0	14.4	226 <sup>a</sup>	225 <sup>a</sup>	244 <sup>b</sup>
<u>Meat quality traits</u>							
Reflectance value, EEL	5.7	6.1*	1	8.1	18.6 <sup>a</sup>	18.4 <sup>a</sup>	19.7 <sup>a</sup>

\*P&lt;0.05; \*\*P&lt;0.01; \*\*\*P&lt;0.001

**Table 1, continued**

<sup>1</sup>Only the traits for which the QTL peak was in the IGF2 region (0-10 cM) and the test statistic [reached]reaching the nominal significance threshold of  $F=3.9$  are included.

<sup>2</sup>"*QTL*" is the test statistic for the presence of a QTL under a genetic model with additive, dominance, and imprinting effects (3 d.f.) while "Imprinting" is the test statistic for the presence of an imprinting effect (1 d.f.), both obtained at the position of the QTL peak. Genome-wise significance thresholds, estimated by permutation, were used for the QTL test while nominal significance thresholds were used for the Imprinting test.

<sup>3</sup>1n cM from the distal end of 2p; IGF2 is located at 0.3 cM.

<sup>4</sup>The reduction in the residual variance of the  $F_2$  population effected by inclusion of an imprinted QTL at the given position.

<sup>5</sup>Means and standard errors estimated at the IGF2 locus by classifying the genotypes according to the population and parent of origin of each allele. *W* and *L* represent alleles derived from the Wild Boar and Large White founders, respectively; [superscript]superscripts *P* and *M* represent a paternal and maternal origin, respectively. Figures with different letters (superscript a or b) are significantly different at least at the 5% level, and most of them are different at the 1% or 0.1% level.

Table 2 Maximum likelihood phenotypic means for the different F2 genotypes estimated under (i) a model of a mendelian QTL, and (ii) a model assuming an imprinted QTL.

Traits	Mendelian QTL				Imprinted QTL		
	$\mu_{LW/LW}$	$\mu_{LW/P}$	$\mu_{P/P}$	R	$\mu_{PAT/LW}$	$\mu_{PAT/P}$	R
BFT (cm)	2.98	2.84	2.64	0.27	2.94	2.70	0.27
% ham	21.10	21.56	22.15	0.83	21.23	21.95	0.83
% loin	24.96	25.53	26.46	0.91	25.12	26.14	0.93
% lean cuts	65.02	65.96	67.60	1.65	65.23	67.05	1.67
% backfat	6.56	6.02	5.33	0.85	6.43	5.56	0.85
% fat cuts	28.92	27.68	26.66	1.46	28.54	26.99	1.49

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**In re Application of:**

ANDERSSON et al.

**Serial No.:** 09/868,732

**Filed:** June 15, 2001

**For:** SELECTING ANIMALS FOR  
PARENTALLY IMPRINTED TRAITS

**Examiner:** To be assigned

**Group Art Unit:** To be assigned

**Attorney Docket No.:** 4951US

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number: EL740548284 US

Date of Deposit with USPS: November 1, 2001

Person making Deposit: Daniel Thatcher

AMENDMENT

Box Non-Fee Amendment  
Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced patent application on the merits, entry of the amendments as set forth herein is respectfully solicited.

Pursuant to 37 C.F.R. §§ 1.121(b)(1)(i), please delete paragraphs [0019], [0021], [0023], [0053], [0054], and [0069] of the substitute specification mailed by the applicants on September 17, 2001, and replace the same with the like-numbered replacement paragraphs [0019], [0021], [0023], [0053], [0054], and [0069] set forth herein. In compliance with 37 C.F.R. §§ 1.121(b)(1)(ii), the above-referenced replacement paragraphs are submitted in clean form below. A version of the replacement paragraphs with markings to show changes made relative to the previous version of such

paragraphs is appended hereto under the title “VERSION WITH MARKINGS TO SHOW CHANGES MADE,” pursuant to 37 C.F.R. §§ 1.121(b)(1)(iii).

IN THE SPECIFICATION:

[0019] Figure 6: Nucleic acid sequences of contig 1 (SEQ ID NO:10), contig 2 (SEQ ID NO:11), contig 3 (SEQ ID NO:12), contig 4 (SEQ ID NO:13), contig 5 (SEQ ID NO:14), contig 6 (SEQ ID NO:15), contig 7 (SEQ ID NO:16), contig 8 (SEQ ID NO:17), contig 9 (SEQ ID NO:18), contig 10 (SEQ ID NO:19), contig 19 (SEQ ID NO:20), contig 20 (SEQ ID NO:21), contig 21 (SEQ ID NO:22), contig 22 (SEQ ID NO:23), contig 23 (SEQ ID NO:24), contig 24 (SEQ ID NO:25), contig 25 (SEQ ID NO:26), contig 26 (SEQ ID NO:27), contig 27 (SEQ ID NO:28), contig 28 (SEQ ID NO:29), contig 29 (SEQ ID NO:30), contig 30 (SEQ ID NO:31), contig 31 (SEQ ID NO:32), contig 32 (SEQ ID NO:33), contig 33 (SEQ ID NO:34), contig 34 (SEQ ID NO:35), contig 35 (SEQ ID NO:36), contig 36 (SEQ ID NO:37), contig 37 (SEQ ID NO:38), contig 38 (SEQ ID NO:39), contig 39 (SEQ ID NO:40), contig 40 (SEQ ID NO:41), contig 41 (SEQ ID NO:42), contig 42 (SEQ ID NO:43), contig 43 (SEQ ID NO:44), contig 44 (SEQ ID NO:45), contig 45 (SEQ ID NO:46), contig 46 (SEQ ID NO:47), contig 47 (SEQ ID NO:48), contig 48 (SEQ ID NO:49), contig 49 (SEQ ID NO:50), contig 50 (SEQ ID NO:51), contig 51 (SEQ ID NO:52), contig 52 (SEQ ID NO:53), contig 53 (SEQ ID NO:54), contig 54 (SEQ ID NO:55), contig 55 (SEQ ID NO:56), contig 56 (SEQ ID NO:57), contig 57 (SEQ ID NO:58), contig 58 (SEQ ID NO:59), contig 59 (SEQ ID NO:60), contig 60 (SEQ ID NO:61), contig 61 (SEQ ID NO:62), contig 62 (SEQ ID NO:63), contig 63 (SEQ ID NO:64), contig 64 (SEQ ID NO:65), contig 65 (SEQ ID NO:66), contig 66 (SEQ ID NO:67), contig 67 (SEQ ID NO:68), contig 68 (SEQ ID NO:69), contig 69 (SEQ ID NO:70), contig 70 (SEQ ID NO:71), contig 71 (SEQ ID NO:72), contig 72 (SEQ ID NO:73), contig 73 (SEQ ID NO:74), contig 74 (SEQ ID NO:75), contig 75 (SEQ ID NO:76), contig 76 (SEQ ID NO:77), contig 77 (SEQ ID NO:78), contig 78 (SEQ ID NO:79), contig 79 (SEQ ID NO:80), contig 80 (SEQ ID NO:81), contig 81 (SEQ ID NO:82), contig 82 (SEQ ID NO:83), contig 83 (SEQ ID NO:84), contig 84 (SEQ ID NO:85), contig 85 (SEQ ID NO:86), contig 86 (SEQ ID NO:87), contig 87 (SEQ ID NO:88), contig 88 (SEQ ID NO:89), contig 89 (SEQ ID NO:90), contig 90 (SEQ ID NO:91), contig 91 (SEQ

ID NO:92), contig 92 (SEQ ID NO:93), contig 93 (SEQ ID NO:94), contig 94 (SEQ ID NO:95), contig 95 (SEQ ID NO:96), contig 96 (SEQ ID NO:97), contig 97, (SEQ ID NO:98), contig 98 (SEQ ID NO:99), contig 99 (SEQ ID NO:100), contig 100 (SEQ ID NO:101), contig 101 (SEQ ID NO:102), contig 102 (SEQ ID NO:103), contig 103 (SEQ ID NO:104), contig 104 (SEQ ID NO:105), contig 105 (SEQ ID NO:106), contig 106 (SEQ ID NO:107), contig 107 (SEQ ID NO:108), contig 108 (SEQ ID NO:109), contig 109 (SEQ ID NO:110), contig 110 (SEQ ID NO:111), contig 111 (SEQ ID NO:112), contig 112 (SEQ ID NO:113), contig 113 (SEQ ID NO:114), contig 114 (SEQ ID NO:115), and contig 115 (SEQ ID NO:116) derived from BAC-PIGF2-1, which was shotgun sequenced using standard procedures and automatic sequencers.

**[0021]** Figure 8: Nucleic acid sequences of contig 1 (SEQ ID NO:117), contig 2 (SEQ ID NO:118), contig 3 (SEQ ID NO:119), contig 4 (SEQ ID NO:120), contig 5 (SEQ ID NO:121), contig 6 (SEQ ID NO:122), and contig 7 (SEQ ID NO:123) derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1), which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

**[0023]** Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals. Polymorphisms 1 through 4 occur in contig 3 (SEQ ID NO:12), polymorphisms 5 through 23 occur in contig 4 (SEQ ID NO: 13), polymorphisms 24 through 28 occur in contig 10 (SEQ ID NO:19), polymorphism 29 occurs in contig 57 (SEQ ID NO:58), polymorphism 20 occurs in contig 95 (SEQ ID NO:96), and polymorphism 31 occurs in contig 105 (SEQ ID NO:106).

**[0053]** Isolation of an IGF2 BAC clone and fluorescent *in situ* hybridisation (FISH). IGF2 primers (F:5'- GGCAAGTTCTTCCGCTAATGA-3' (SEQ ID NO:1) and R:5' - GCACCGCAGAATTACGACAA-3' (SEQ ID NO:2)) for PCR amplification of a part of the last exon and 3'UTR were designed on the basis of a porcine IGF2 cDNA sequence (GenBank X56094). The primers were used to screen a porcine BAC library and the clone 253G10 was isolated. Crude BAC DNA was prepared as described<sup>24</sup>. The BAC DNA was linearized with *EcoRV* and purified

with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two days at room temperature and then kept at -20°C until use. FISH analysis was carried out as previously described<sup>25</sup>. The final concentration of the probe in the hybridisation mix was 10 ng/ $\mu$ l. Repetitive sequences were suppressed with standard concentrations of porcine genomic DNA. After post-hybridisation washing, the biotinylated probe was detected with two layers of avidin-FITC (Vector A-2011). The chromosomes were counterstained with 0.3 mg/ml DAPI (4, 6-Diamino-2-phenylindole; Sigma D9542), which produced a G-banding like pattern. No posthybridisation banding was needed, since chromosome 2 is easily recognized without banding. A total of 20 metaphase spreads were examined under an Olympus BX-60 fluorescence microscope connected to an IMAC-CCD S30 video camera and equipped with an ISIS 1.65 (Metasystems) software.

**[0054]** About two  $\mu$ g of linearized and purified BAC DNA was used for direct sequencing with 20 pmoles of primers and BigDye Terminator chemistry (Perkin Elmer, USA). DNA sequencing was done from the 3' end of the last exon towards the 3' end of the UTR until a microsatellite was detected. A primer set (F:5'-GTTTCTCCTGTACCCACACGCATCCC-3' (SEQ ID NO:3) and R: 5'-Fluorescein-CTACAAGCTGGGCTCAGGG-3' (SEQ ID NO:4)) was designed for the amplification of the IGF2 microsatellite which is about 250 bp long and located approximately 800 bp downstream from the stop codon. The microsatellite was PCR amplified using fluorescently labeled primers and the genotyping was carried out using an ABI377 sequencer and the GeneScan/Genotyper softwares (Perkin Elmer, USA). Two-point and multipoint linkage analyses were done with the Cri-Map software<sup>26</sup>.

**[0069]** *Marker genotyping:* Primer pairs utilised for PCR amplification of microsatellite markers are as described<sup>19</sup>. Marker genotyping was performed as previously described<sup>20</sup>. Genotypes at the *CRC* and *MyoD* loci were determined using conventional methods as described<sup>1,12</sup>. The LAR test for the IGF2 SNP was developed according to Baron et al.<sup>21</sup> using a primer pair for PCR

amplification (5'-CCCCTGAACTTGAGGACGAGCAGCC-3'(SEQ ID NO:5); 5'-ATCGCTGTGGGCTGGGTGGGCTGCC-3')(SEQ ID NO:6) and a set of three primers for the LAR step (5'-FAM-CGCCCCAGCTGCCCCCAG-3'(SEQ ID NO:7); 5' -HEX-CGCCCCAGCTGCCCCCAA-3'(SEQ ID NO:8); 5' -CCTGAGCTGCAGCAGGCCAG-3' (SEQ ID NO:9)).



**REMARKS**

No new matter has been added. The Applicants again request entry of the amendments as set forth herein prior to examination of the application on the merits.

Respectfully submitted,



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Date: November 1, 2001

## VERSION WITH MARKINGS TO SHOW CHANGES MADE

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Title: Selecting animals for parentally imprinted traits.

5

The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition. Breeding schemes for domestic animals have so far focused on farm performance traits and carcass quality. This has resulted in substantial improvements in traits like reproductive success, milk production, lean/fat ratio, prolificacy, growth rate and feed efficiency. Relatively simple performance test data have been the basis for these improvements, and selected traits were assumed to be influenced by a large number of genes, each of small effect (the infinitesimal gene model). There are now some important changes occurring in this area. First, the breeding goal of some breeding organisations has begun to include meat quality attributes in addition to the "traditional" production traits. Secondly, evidence is accumulating that current and new breeding goal traits may involve relatively large effects (known as major genes), as opposed to the infinitesimal model that has been relied on so far.

Modern DNA-technologies provide the opportunity to exploit these major genes, and this approach is a very promising route for the improvement of meat quality, especially since direct meat quality assessment is not viable for potential breeding animals. Also for other traits such as lean/fat ratio, growth rate and feed efficiency, modern DNA technology can be very effective. Also these traits are not always easy to measure in the living animal.

The evidence for several of the major genes originally obtained using segregation analysis, i.e. without any DNA marker information. Afterwards molecular studies were performed to detect the location of these

genes on the genetic map. In practice, and except for alleles of very large effect, DNA studies are required to dissect the genetic nature of most traits of economic importance. DNA markers can be used to localise genes or alleles responsible for qualitative traits like coat colour, and they can also be used to detect genes or alleles with substantial effects on quantitative traits like growth rate, IMF etc. In this case the approach is referred to as QTL (quantitative trait locus) mapping, wherein a QTL comprises at least a part of the nucleic acid genome of an animal where genetic information capable of influencing said quantitative trait (in said animal or in its offspring) is located. Information at DNA level can not only help to fix a specific major gene in a population, but also assist in the selection of a quantitative trait which is already selected for. Molecular information in addition to phenotypic data can increase the accuracy of selection and therefore the selection response.

Improving meat quality or carcass quality is not just about changing levels of traits like tenderness or marbling, but it is also about increasing uniformity. The existence of major genes provides excellent opportunities for improving meat quality because it allows large steps to be made in the desired direction. Secondly, it will help to reduce variation, since we can fix relevant genes in our products. Another aspect is that selecting for major genes allows differentiation for specific markets. Studies are underway in several species, particularly, pigs, sheep, deer and beef cattle.

In particular, intense selection for meat production has resulted in animals with extreme muscularity and leanness in several livestock species. In recent years it has become feasible to map and clone several of the genes causing these phenotypes, paving the way towards more efficient marker assisted selection, targeted drug development (performance enhancing products) and transgenesis. Mutations in the ryanodine receptor (Fuji

et al, 1991; MacLennan and Phillips, 1993) and myostatin (Grobet et al, 1997; Kambadur et al, 1997; McPherron and Lee, 1997) have been shown to cause muscular hypertrophies in pigs and cattle respectively, while  
5 genes with major effects on muscularity and/or fat deposition have for instance been mapped to pig chromosome 4 (Andersson et al, 1994) and sheep chromosome 18 (Cocket et al, 1996).

However, although there have been successes in  
10 identifying QTLs, the information is currently of limited use within commercial breeding programmes. Many workers in this field conclude that it is necessary to identify the particular genes underlying the QTL. This is a substantial task, as the QTL region is usually relatively  
15 large and may contain many genes. Identification of the relevant genes from the many that may be involved thus remains a significant hurdle in farm animals.

The invention provides a method for selecting a  
20 domestic animal for having desired genotypic or potential phenotypic properties comprising testing said animal for the presence of a parentally imprinted qualitative or quantitative trait locus (QTL). Herein, a domestic animal is defined as an animal being selected or having been  
25 derived from an animal having been selected for having desired genotypic or potential phenotypic properties.

Domestic animals provide a rich resource of genetic and phenotypic variation, traditionally domestication involves selecting an animal or its offspring for having  
30 desired genotypic or potential phenotypic properties. This selection process has in the past century been facilitated by growing understanding and utilisation of the laws of Mendelian inheritance. One of the major problems in breeding programs of domestic animals is the  
35 negative genetic correlation between reproductive capacity and production traits. This is for example the case in cattle (a high milk production generally results



in slim cows and bulls) poultry, broiler lines have a low level of egg production and layers have generally very low muscle growth), pigs (very prolific sows are in general fat and have comparatively less meat) or sheep

5 (high prolific breeds have low carcass quality and vice versa). The invention now provides that knowledge of the parental imprinting character of various traits allows to select for example sire lines homozygous for a paternally imprinted QTL for example linked with muscle production

10 or growth; the selection for such traits can thus be less stringent in dam lines in favour of the reproductive quality. The phenomenon of genetic or parental imprinting has never been utilised in selecting domestic animals, it was never considered feasible to employ this elusive

15 genetic characteristic in practical breeding programmes. The invention provides a breeding programme, wherein knowledge of the parental imprinting character of a desired trait, as demonstrated herein, results in a breeding programme, for example in a BLUP programme, with

20 a modified animal model. This increases the accuracy of the breeding value estimation and speeds up selection compared to conventional breeding programmes. Until now, the effect of a parentally imprinted trait in the estimation of a conventional BLUP programme was

25 neglected; using and understanding the parental character of the desired trait, as provided by the invention, allows selecting on parental imprinting, even without DNA testing. For example, selecting genes characterised by paternal imprinting is provided to help increase

30 uniformity; a (terminal) parent homozygous for the "good or wanted" alleles will pass them to all offspring, regardless of the other parent's alleles, and the offspring will all express the desired parent's alleles. This results in more uniform offspring. Alleles that are

35 interesting or favourable from the maternal side or often the ones that have opposite effects to alleles from the paternal side. For example, in meat animals such as pigs alleles linked with meat quality traits such as intra-

muscular fat or muscle mass could be fixed in the dam lines while alleles linked with reduced back fat could be fixed in the sire lines. Other desirable combinations are for example fertility and/or milk yield in the female  
5 line with growth rates and/or muscle mass in the male lines.

In a preferred embodiment, the invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising  
10 testing a nucleic acid sample from said animal for the presence of a parentally imprinted quantitative trait locus (QTL). A nucleic acid sample can in general be obtained from various parts of the animal's body by methods known in the art. Traditional samples for the  
15 purpose of nucleic acid testing are blood samples or skin or mucosal surface samples, but samples from other tissues can be used as well, in particular sperm samples, oocyte or embryo samples can be used. In such a sample, the presence and/or sequence of a specific nucleic acid,  
20 be it DNA or RNA, can be determined with methods known in the art, such as hybridisation or nucleic acid amplification or sequencing techniques known in the art. The invention provides testing such a sample for the presence of nucleic acid wherein a QTL or allele  
25 associated therewith is associated with the phenomenon of parental imprinting, for example where it is determined whether a paternal or maternal allele of said QTL is capable of being predominantly expressed in said animal.

The purpose of breeding programs in livestock is to  
30 enhance the performances of animals by improving their genetic composition. In essence this improvement accrues by increasing the frequency of the most favourable alleles for the genes influencing the performance characteristics of interest. These genes are referred to  
35 as QTL. Until the beginning of the nineties, genetic improvement was achieved via the use of biometrical methods, but without molecular knowledge of the underlying QTL.

Since the beginning of the nineties and due to recent developments in genomics, it is conceivable to identify the QTL underlying a trait of interest. The invention now provides identifying and using parentally  
5 imprinted QTLs which are useful for selecting animals by mapping quantitative trait loci. Again, the phenomenon of genetic or paternal imprinting has never been utilised in selecting domestic animals, it was never considered  
feasible to employ this elusive genetic characteristic in  
10 practical breeding programmes. For example Kovacs and Kloting (Biochem. Mol. Biol. Int. 44:399-405, 1998), where parental imprinting is not mentioned, and not suggested, found linkage of a trait in female rats, but not in males, suggesting a possible sex specificity  
15 associated with a chromosomal region, which of course excludes parental imprinting, a phenomenon wherein the imprinted trait of one parent is preferably but gender-aspecifically expressed in his or her offspring.

The invention provides the initial localisation of a  
20 parentally imprinted QTL on the genome by linkage analysis with genetic markers, and the actual identification of the parentally imprinted gene(s) and causal mutations therein. Molecular knowledge of such a parentally imprinted QTL allows for more efficient  
25 breeding designs herewith provided. Applications of molecular knowledge of parentally imprinted QTLs in breeding programs include: marker assisted segregation analysis to identify the segregation of functionally distinct parentally imprinted QTL alleles in the  
30 populations of interest, marker assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy, selection intensity or by reducing the generation interval using the understanding of the phenomenon of parental imprinting, marker assisted  
35 introgression (MAI) to efficiently transfer favourable parentally imprinted QTL alleles from a donor to a recipient population, genetic engineering of the identified parentally QTL and genetic modification of the breeding stock using transgenic technology, development

of performance enhancing products using targeted drug development exploiting molecular knowledge of said QTL.

The inventors undertook two independent experiments to determine the practical use of parental imprinting of a QTL.

In a first experiment, performed in a previously described Piétrain x Large White intercross, the likelihood of the data were computed under a model of paternal (paternal allele only expressed) and maternal imprinting (maternal allele only expressed) and compared with the likelihood of the data under a model of a conventional "Mendelian" QTL. The results strikingly demonstrated that the QTL was indeed paternally expressed, the QTL allele (Piétrain or Large White) inherited from the F<sub>1</sub> sow having no effect whatsoever on the carcass quality and quantity of the F<sub>2</sub> offspring. It was seen that very significant lodscores were obtained when testing for the presence of a paternally expressed QTL, while there was no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. The same tendency was observed for all traits showing that the same imprinted gene is responsible for the effects observed on the different traits. Table 1 reports the maximum likelihood (ML) phenotypic means for the F<sub>2</sub> offspring sorted by inherited paternal QTL allele.

In a second experiment performed in the Wild Boar X Large White intercross, QTL analyses of body composition, fatness, meat quality, and growth traits was carried out with the chromosome 2 map using a statistical model testing for the presence of an imprinting effect. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; Table1). The clear paternal expression of a QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). For a given paternally imprinted QTL, implementation of marker assisted segregation analysis, selection (MAS) and introgression (MAI), can be performed

using genetic markers that are linked to the QTL, genetic markers that are in linkage disequilibrium with the QTL, or using the actual causal mutations within the QTL.

Understanding the parent-of-origin effect

- 5 characterising a QTL allows for its optimal use in breeding programs. Indeed, marker assisted segregation analysis under a model of parental imprinting will yield better estimates of QTL allele effects. Moreover it allows for the application of specific breeding schemes
- 10 to optimally exploit a QTL. In one embodiment of the invention, the most favourable QTL alleles would be fixed in breeding animal lines and for example used to generate commercial, crossbred males by marker assisted selection (MAS, within lines) and marker assisted introgression
- 15 (MAI, between lines). In another embodiment, the worst QTL alleles would be fixed in the animal lines used to generate commercial crossbred females by MAS (within lines) and MAI (between lines).

- In a preferred embodiment of the invention, said
- 20 animal is a pig. Note for example that the invention provides the insight that today half of the offspring from commercially popular Piétrain<sub>x</sub> Large White crossbred boars inherit an unfavourable Large White muscle mass QTL as provided by the invention causing considerable loss,
- 25 and the invention now for example provides the possibility to select the better half of the population in that respect. However, it is also possible to select commercial sow lines enriched with the in the boars unfavourable alleles, allowing to equip the sows with
- 30 other alleles more desirable for for example reproductive purposes.

- In a preferred embodiment of a method provided by the invention, said QTL is located at a position corresponding to a QTL located at chromosome 2 in the
- 35 pig. For example, it is known from comparative mapping data between pig and human, including bidirectional chromosome painting, that SSC2p is homologous to HSA11pter-q13<sup>11,12</sup>. HSA11pter-q13 is known to harbour a

cluster of imprinted genes: IGF2, INS2, H19, MAH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, Tapal,/CD81, Orctl2, Impt1 and Ip1. The cluster of imprinted genes located in HSA11pter-q13 is characterised by 8 maternally expressed genes H19, MASH2, P57<sup>KIP2</sup>, K<sub>v</sub>LQTL1, TAPAl/CD81, ORCTL2, IMPT1 and IP1, and two paternally expressed genes: IGF2 and INS. However, Johanson et al (Genomics 25:682-690, 1995) and Reik et al (Trends in Genetics, 13:330-334, 1997) show that the whereabouts of these loci in various animals are not clear. For example, the HSA11 and MMU7 loci do not correspond among each other, the MMU7 and the SSC2 loci do not correspond, whereas the HSA11 and SSC2 loci seem to correspond, and no guidance is given where one or more of for example the above identified parentally expressed individual genes are localised on the three species' chromosomes.

Other domestic animals, such as cattle, sheep, poultry and fish, having similar regions in their genome harbouring such a cluster of imprinted genes or QTLs, the invention herewith provides use of these orthologous regions of other domestic animals in applying the phenomenon of parental imprinting in breeding programmes. In pigs, said cluster is mapped at around position 2p1.7 of chromosome 2, however, a method as provided by the invention employing (fragments of) said maternally or paternally expressed orthologous or homologous genes or QTLs are advantageously used in other animals as well for breeding and selecting purposes. For example, a method is provided wherein said QTL is related to the potential muscle mass and/or fat deposition, preferably with limited effects on other traits such as meat quality and daily gain of said animal or wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) allele. Reik et al (Trends in Genetics, 13:330-334, 1997) explain that this gene in humans is related to Beckwith-Wiedemann syndrome, an apparently parentally imprinted disease syndrome most commonly seen with human fetuses, where the gene has an important role in prenatal

development. No relationship is shown or suggested with postnatal development relating to muscle development or fatness in (domestic) animals.

In a preferred embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7. In particular, the invention relates to the use of genetic markers for the telomeric end of pig chromosome 2p in marker selection (MAS) of a parentally imprinted Quantitative Trait Locus (QTL) affecting carcass yield and quality in pigs. Furthermore, the invention relates to the use of genetic markers associated with the IGF2 locus in MAS in pigs, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. In a preferred embodiment, the invention provides a QTL located at the distal tip of *Sus scrofa* chromosomes 2 with effects on varies measurements of carcass quality and quantity, particularly muscle mass and fat deposition.

In a first experiment, a QTL mapping analysis was performed in a Wild Boar X Large White intercross counting 200 F<sub>2</sub> individuals. The F<sub>2</sub> animals were sacrificed at a live eight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are provided by Andersson et al<sup>1</sup> and Andersson-Eklund et al<sup>4</sup>.

A QTL (without any significant effect on back-fat thickness) at an unspecified locus on the proximal end of chromosome 2 with moderate effect on muscle mass, and located about 30cM away from the parentally imprinted QTL reported here, was previously reported by the inventors; whereas the QTL as now provided has a very large effect, explaining at least 20-30% of variance, making the QTL of

the present invention commercially very attractive, which is even more so because the present QTL is parentally imprinted. The marker map of chromosome 2p was improved as part of this invention by adding microsatellite markers in order to cover the entire chromosome arm. The following microsatellite markers were used: *Swc9*, *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map. Clear evidence for a QTL located at the very distal tip of 2p was obtained (Fig. 1; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population.

In a second experiment, QTL mapping was performed in a Piétrain X Large White intercross comprising 1125 F<sub>2</sub> offspring. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famous for their exceptional muscularity and leanness<sup>10</sup> (Figure 2, while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring growth performance (5), muscularity (6), fat deposition (6), and meat quality (4), were recorded on all F<sub>2</sub> offspring. In order to map QTL underlying the genetic differences between these breeds, the inventors undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. The following microsatellite marker map was used to analyse



chromosome 2;:SW2443, SWC9 and SW2623, SWR2516-(0,20)-  
SWR783-(0,29)-SW240-(0,20)-SW776-(0,08)-S0010-(0,04)-  
SW1695-(0,36)-SWR308. Analysis of pig chromosome 2 using  
a Maximum Likelihood multipoint algorithm, revealed  
5 highly significant lodscores (up to 20) for three of the  
six phenotypes measuring muscularity (% lean cuts, % ham,  
% loin) and three of the six phenotypes measuring fat  
deposition (back-fat thickness (BFT), % backfat, % fat  
cuts) at the distal end of the short arm of chromosome 2  
10 (Figure 1). Positive lodscores were obtained in the  
corresponding chromosome region for the remaining six  
muscularity and fatness phenotypes, however, not reaching  
the experiment-wise significance threshold ( $\alpha=5\%$ ). There  
was no evidence for an effect of the corresponding QTL on  
15 growth performance (including birth weight) or recorded  
meat quality measurements (data not shown). To confirm  
this finding, the remaining sample of 355  $F_2$  offspring was  
genotyped for the four most distal 2p markers and QTL  
analysis performed for the traits yielding the highest  
20 lodscores in the first analysis. Lodscores ranged from  
2.1 to 7.7, clearly confirming the presence of a major  
QTL in this region. Table 2 reports the corresponding ML  
estimates for the three genotypic means as well as the  
residual variance. Evidence based on marker assisted  
25 segregation analysis points towards residual segregation  
at this locus within the Piétrain population.

These experiments therefore clearly indicated  
the existence of a QTL with major effect on carcass  
quality and quantity on the telomeric end of pig  
30 chromosome arm 2p; the likely existence of an allelic  
series at this QTL with at least three alleles: Wild-Boar  
< Large White < Piétrain, and possibly more given the  
observed segregation within the Piétrain breed.

The effects of the identified QTL on muscle mass and  
35 fat deposition are truly major, being of the same  
magnitude of those reported for the CRC locus though  
apparently without the associated deleterious effects on  
meat quality. We estimate that both loci jointly explain

close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the F<sub>2</sub> population. Large effects on the area of the longissimus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL, when compared to the Wild Boar allele, was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits shows that a single causative locus is involved. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output.

In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7., wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele or a genonic area closely related thereto, such as polymorphisms and microsatellites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. The important role of *IGF2* for prenatal development is well-documented from knock-out mice as well as from its causative role in the human Beckwith-Wiedemann syndrome. This invention demonstrates an important role for the *IGF2*-region also for postnatal development.

To show the role of Igf2 the inventors performed the following three experiments:

A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone  
5 gave a strong consistent signal on the terminal part of chromosome 2p.

A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible  
10 presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800bp downstream of the stop codon; a sequence comparison revealed that this microsatellite was  
15 identical to a previously described anonymous microsatellite, Swc9<sup>6</sup>. This marker was used in the initial QTL mapping experiments and its location on the genetic map correspond with the most likely position of the QTL both in the Piétrain X Large White and in the Large White  
20 x Wild Boar pedigree.

Analysis of skeletal muscle and liver cDNA from 10-week old fetuses heterozygous for a nt241 (G-A) transversion in the second exon of the porcine IGFII gene and SWC9, shows that the IGFII gene is imprinted in these  
25 tissues in the pig as well and only expressed from the paternal allele.

Based on a published porcine adult liver cDNA sequence<sup>16</sup>, the inventors designed primer pairs allowing to amplify the entire *IgfII* coding sequence with 222 bp  
30 of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced indicating that the coding sequences are identical in both breeds and with the published sequence. However, a G→A transition was found  
35 in the leader sequence corresponding to exon 2 in man. Following conventional nomenclature, this polymorphism will be referred to as nt241(G-A). We developed a screening test for this single nucleotide polymorphism

9(SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, *IgfII* was shown to colocalize with the SWC9 microsatellite marker ( $\theta=0\%$ ), therefore  
5 virtually coinciding with the most likely position of the QTL, and well within the 95% support interval for the QTL. Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3'UTR of the *IgfII* gene.

10 As previously mentioned, the knowledge of this QTL provides a method for the selection of animals such as pigs with improved carcass merit. Different embodiments of the invention are envisaged, including:  
15 segregation of functionally distinct QTL alleles in the populations of interest; marker assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy, selection intensity or by reducing the generation interval; marker assisted  
20 introgression (MAI) to efficiently transfer favourable QTL alleles from a donor to a recipient population, thereby enhancing genetic response in the recipient population. Implementation of embodiments marker assisted segregation analysis, selection (MAS) and introgression  
25 (MAI), can be performed using genetic markers that are linked to the QTL; genetic markers that are in linkage disequilibrium with the QTL, the actual causal mutations within the QTL.

In a further embodiment, the invention provides a  
30 method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7., wherein said QTL is  
35 paternally expressed, i.e. is expressed from the paternal allele. In man and mouse, *Igf2* is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues. Analysis of skeletal muscle cDNA from

pigs heterozygous for the SNP and/or SWC9, shows that the same imprinting holds in the pig as well. Understanding the parent-of-origin effect characterising the QTL as provided by the invention now allows for its optimal use in breeding programs. Indeed, today half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing considerable loss. Using a method as provide by the invention avoids this problem.

10 The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof comprising a parentally imprinted quantitative trait locus (QTL) or fragment thereof capable of being predominantly expressed by one parental  
15 allele. Having such a nucleic acid as provided by the invention available allows constructing transgenic animals wherein favourable genes are capable of being exclusively or predominantly expressed by one parental allele, thereby equipping the offspring of said animal  
20 homozygous for a desired trait with desired properties related to that parental allele that is expressed.

In a preferred embodiment, the invention provides an isolated and/or recombinant nucleic acid or fragment derived thereof comprising a synthetic parentally  
25 imprinted quantitative trait locus (QTL) or functional fragment thereof derived from at least one chromosome. Synthetic herein describes a parentally expressed QTL wherein various elements are combined that originate from distinct locations from the genome of one or more  
30 animals. The invention provides recombinant nucleic acid wherein sequences related to parental imprinting of one QTL are combined with sequences relating to genes or favourable alleles of a second QTL. Such a gene construct is favourably used to obtain transgenic animals wherein  
35 the second QTL has been equipped with paternal imprinting, as opposed to the inheritance pattern in the native animal from which the second QTL is derived. Such a second QTL can for example be derived from the same

chromosome where the parental imprinting region is located, but can also be derived from a different chromosome from the same or even a different species. In the pig, such a second QTL can for example be related to an oestrogen receptor (ESR)-gene (Rothschild et al, PNAS, 93, 201-201, 1996) or a FAT-QTL (Andersson, Science, 263, 1771-1774, 1994) for example derived from an other pig chromosome, such as chromosome 4. A second or further QTL can also be derived from another (domestic) animal or a human.

The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof at least partly corresponding to a QTL of a pig located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7 wherein said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or wherein said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele, preferably at least spanning a region between INS and H19, or preferably derived from a domestic pig, such as a Pietrain, Meishan, Duroc, Landrace or Large White, or from a Wild Boar. For example, a genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p. A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, Swc9. PCR primers were designed and the microsatellite (IGF2ms) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and another two

among the eight Large White founders. *IGF2ms* was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each F<sub>2</sub> animal.

5        A linkage analysis using the intercross pedigree was carried out with *IGF2ms* and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. *IGF2* was firmly assigned to 2p by highly significant lod scores (e.g. Z=89.0,  $\theta=0.003$  against *Swr2516*). Multipoint  
10 analyses, including previously typed chromosome 2 markers, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443/Swr2516*-0.3-*IGF2*-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed  
15 location of *IGF2* in relation to these loci is based on a single recombinant giving a lod score support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

20        The invention furthermore provides use of a nucleic acid or functional fragment derived thereof according to the invention in a method according to the invention. In a preferred embodiment, use of a method according to invention is provided to select a breeding animal or  
25 animal destined for slaughter, or embryos or semen derived from these animals for having desired genotypic or potential phenotypic properties. In particular, the invention provides such use wherein said properties are related to muscle mass and/or fat deposition. The QTL as  
30 provided by the invention may be exploited or used to improve for example lean meat content or back-fat thickness by marker assisted selection within populations or by marker assisted introgression of favorable alleles from one population to another. Examples of marker  
35 assisted selection using the QTL as provided by the invention are use of marker assisted segregation analysis

with linked markers or with markers in disequilibrium to identify functionally distinct QTL alleles. Furthermore, identification of a causative mutation in the QTL is now possible, again leading to identify functionally distinct QTL alleles. Such functionally distinct QTL alleles located at the distal tip of chromosome 2p with large effects on skeletal muscle mass, the size of the heart, and on back-fat thickness are also provided by the invention. The observation of a similar QTL effect in a Large White x Wild Boar as well as in a Piétrain x Large White intercross provides proof of the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on marker assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series as provided by the invention allows identifying causal polymorphisms which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found i.e. Piétrain pigs are more muscular than Large White pigs that in turn have higher lean meat content than Wild Boars. The invention furthermore provides use of the alleles as provided by the invention for within line selection or for marker assisted introgression using linked markers, markers in disequilibrium or alleles comprising causative mutations.

The invention furthermore provides an animal selected by using a method according to the invention. For example, a pig characterised in being homozygous for an allele in a QTL located at a *Sus scrofa* chromosome 2 mapping at position 2p1.7 can now be selected and is thus provided by the invention. Since said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or said QTL comprises at least a part of a *Sus scrofa* insulin-like growth factor-2 (IGF2) allele, it is



possible to select promising pigs to be used for breeding or to be slaughtered. In particular an animal according to the invention which is a male is provided. Such a male, or its sperm or an embryo derived thereof can advantageously be used in breeding animals for creating breeding lines or for finally breeding animals destined for slaughter. In a preferred embodiment of such use as provided by the invention, a male, or its sperm, deliberately selected for being homozygous for an allele causing the extreme muscular hypertrophy and leanness, is used to produce offspring heterozygous for such an allele. Due to said allele's paternal expression, said offspring will also show the favourable traits for example related to muscle mass, even if the parent female has a different genetic background. Moreover, it is now possible to positively select the female(s) for having different traits, for example related to fertility, without having a negative effect on the muscle mass trait that is inherited from the allele from the selected male. For example, earlier such males could occasionally be seen with Piétrain pigs but genetically it was not understood how to most profitably use these traits in breeding programmes.

Furthermore, the invention provides a transgenic animal, sperm and an embryo derived thereof, comprising a synthetic parentally imprinted QTL or functional fragment thereof as provided by the invention, i.e. it is provided by the invention to introduce a favourable recombinant allele; for example introduce the oestrogen receptor locus related to increased litter size of an animal homozygously in a parentally imprinted region of a grandparent animal (for example the father of a hybrid sow if the region was paternally imprinted and the grandparent was a boar); to introduce a favourable fat-related allele or muscle mass-related recombinant allele in a paternally imprinted region, and so on. Recombinant alleles that are interesting or favourable from the maternal side or often the ones that have opposite effects to alleles from the paternal side. For example,

in meat animals such as pigs recombinant alleles linked with meat quality traits such as intra-muscular fat or muscle mass could be fixed in the dam lines while recombinant alleles linked with reduced back fat could be fixed in the sire lines. Other desirable combinations are for example fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

The invention is further explained in the detailed description without limiting the invention.

Detailed description.

#### Example 1: Wild Boar x Large White intercrosses

##### Methods

Isolation of an *IGF2* BAC clone and fluorescent *in situ* hybridization (FISH). *IGF2* primers (F:5'-GGCAAGTTCTTCCGCTAATGA-3' and R:5'-GCACCGCAGAATTACGACAA-3') for PCR amplification of a part of the last exon and 3'UTR were designed on the basis of a porcine *IGF2* cDNA sequence (GenBank X56094). The primers were used to screen a porcine BAC library and the clone 253G10 was isolated. Crude BAC DNA was prepared as described<sup>24</sup>. The BAC DNA was linearized with *EcoRV* and purified with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two days at room temperature and then kept at -20°C until use. FISH analysis was carried out as previously described<sup>25</sup>. The final concentration of the probe in the hybridization mix was 10 ng/μl. Repetitive sequences were suppressed with standard concentrations of porcine

genomic DNA. After post-hybridization washing, the biotinylated probe was detected with two layers of avidin-FITC (Vector A-2011). The chromosomes were counterstained with 0.3 mg/ml DAPI (4,6-Diamino-2-phenylindole; Sigma D9542), which produced a G-banding like pattern. No posthybridization banding was needed, since chromosome 2 is easily recognized without banding. A total of 20 metaphase spreads were examined under an Olympus BX-60 fluorescence microscope connected to an IMAC-CCD S30 video camera and equipped with an ISIS 1.65 (Metasystems) software.

Sequence, microsatellite, and linkage analysis.

About two  $\mu$ g of linearized and purified BAC DNA was used for direct sequencing with 20 pmoles of primers and BigDye Terminator chemistry (Perkin Elmer, USA). DNA sequencing was done from the 3' end of the last exon towards the 3' end of the UTR until a microsatellite was detected. A primer set (F:5'-GTTTCTCCTGTACCCACACGCATCCC-3' and R:5'-Fluorescein-CTACAAGCTGGGCTCAGGG-3') was designed for the amplification of the *IGF2* microsatellite which is about 250 bp long and located approximately 800 bp downstream from the stop codon. The microsatellite was PCR amplified using fluorescently labeled primers and the genotyping was carried out using an ABI377 sequencer and the GeneScan/Genotyper softwares (Perkin Elmer, USA). Two-point and multipoint linkage analysis were done with the Cri-Map software<sup>26</sup>.

Animals and phenotypic data.

The intercross pedigree comprised two European Wild Boar males and eight Large White females, 4 F<sub>1</sub> males and 22 F<sub>1</sub> females, and 200 F<sub>2</sub> progeny<sup>1</sup>. The F<sub>2</sub> animals were sacrificed at a live weight of at least 80 kg or at a

maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are  
5 provided by Andersson *et al.*<sup>1</sup> and Andersson-Eklund *et al.*<sup>4</sup>

Statistical analysis.

10 Interval mapping for the presence of QTL were carried out with a least squares method developed for the analysis of crosses between outbred lines<sup>27</sup>. The method is based on the assumption that the two divergent lines are fixed for alternative QTL alleles. There are four possible  
15 genotypes in the F<sub>2</sub> generation as regards the grandparental origin of the alleles at each locus. This makes it possible to fit three effects: additive, dominance, and imprinting<sup>2</sup>. The latter is estimated as the difference between the two types of heterozygotes,  
20 the one receiving the Wild Boar allele through an F<sub>1</sub> sire and the one receiving it from an F<sub>1</sub> dam. An F-ratio was calculated using this model (with 3 d.f.) versus a reduced model without a QTL effect for each cM of chromosome 2. The most likely position of a QTL was  
25 obtained as the location giving the highest F-ratio. Genome-wise significance thresholds were obtained empirically by a permutation test<sup>28</sup> as described<sup>2</sup>. The QTL model including an imprinting effect was compared with a model without imprinting (with 1 d.f.) to test  
30 whether the imprinting effect was significant.

The statistical models also included the fixed effects and covariates that were relevant for the respective traits; see Andersson-Eklund *et al.*<sup>4</sup> for a more detailed description of the statistical models used.  
35 Family was included to account for background genetic

effects and maternal effects. Carcass weight was included as a covariate to discern QTL effects on correlated traits, which means that all results concerning body composition were compared at equal weights. Least-squares means for each genotype class at the *IGF2* locus were estimated with a single point analysis using Procedure GLM of SAS<sup>29</sup>; the model included the same fixed effects and covariates as used in the interval mapping analyses. The QTL shows a clear parent of origin-specific expression and the map position coincides with that of the insulin-like growth factor II gene (*IGF2*), indicating *IGF2* as the causative gene. A highly significant segregation distortion (excess of Wild Boar-derived alleles) was also observed at this locus. The results demonstrate an important effect of the *IGF2* region on postnatal development and it is possible that the presence of a paternally expressed *IGF2*-linked QTL in humans and in rodent model organisms has so far been overlooked due to experimental design or statistical treatment of data. The study has also important implications for quantitative genetics theory and practical pig breeding.

*IGF2* was identified as a positional candidate gene for this QTL due to the observed similarity between pig chromosome 2p and human chromosome 11p. A genomic *IGF2* clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p (Fig. 1). A polymorphic microsatellite is located in the 3'UTR of *IGF2* in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the *IGF2* 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical

to a previously described anonymous microsatellite, *Swc96*. PCR primers were designed and the microsatellite (*IGF2ms*) was found to be highly polymorphic with three different alleles among the two Wild Boar founders and  
5 another two among the eight Large White founders. *IGF2ms* was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with confidence for each allele in each  $F_2$  animal.

10 A linkage analysis using the intercross pedigree was carried out with *IGF2ms* and the microsatellites *Sw2443*, *Sw2623*, and *Swr2516*, all from the distal end of 2p<sup>7</sup>. *IGF2* was firmly assigned to 2p by highly significant lod scores (e.g.  $Z=89.0$ ,  $\theta=0.003$  against *Swr2516*). Multipoint  
15 analyses, including previously typed chromosome 2 markers<sup>8</sup>, revealed the following order of loci (sex-average map distances in Kosambi cM): *Sw2443/Swr2516*-0.3-*IGF2*-14.9-*Sw2623*-10.3-*Sw256*. No recombinant was observed between *Sw2443* and *Swr2516*, and the suggested proximal  
20 location of *IGF2* in relation to these loci is based on a single recombinant giving a lod score support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

25 QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 map using a statistical model testing for the possible presence of an imprinting effect as expected for *IGF2*. Clear evidence for a paternally expressed QTL  
30 located at the very distal tip of 2p was obtained (Fig. 2; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in the  $F_2$  population. Large effects on the area of the longissimus dorsi muscle, on  
35 the weight of the heart, and on back-fat thickness

(subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits strongly suggests a single causative locus. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point of view as a larger muscle mass requires a larger cardiac output. The clear paternal expression of this QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). It is worth noticing though that there was a non-significant trend towards less extreme values for the two heterozygous classes, in particular for the estimated effect on the area of longissimus dorsi. This may be due to chance, but could have a biological explanation, e.g. that there is some expression of the maternally inherited allele or that there is a linked, non-imprinted QTL with minor effects on the traits in question.

The *IGF2*-linked QTL and the *FAT1* QTL on chromosome 4<sup>1, 9</sup> are by far the two loci with the largest effect on body composition and fatness segregating in this Wild Boar intercross. The *IGF2* QTL controls primarily muscle mass whereas *FAT1* has major effects on fat deposition including abdominal fat, a trait that was not affected by the *IGF2* QTL (Fig. 2). No significant interaction between the two loci was indicated and they control a very large proportion of the residual phenotypic variance in the F<sub>2</sub> generation. A model including both QTLs explains 33.1% of the variance for percentage lean meat in ham, 31.3% for the percentage of lean meat plus bone in back, and 26.2%

for average back fat depth (compare with a model including only chromosome 2 effects, Table 1). The two QTLs must have played a major role in the response during selection for lean growth and muscle mass in the Large White domestic pig.

A highly significant segregation distortion was observed in the *IGF2* region (excess of Wild Boar-derived alleles) as shown in Table 1 ( $\chi^2=11.7$ , d.f.=2;  $P=0.003$ ). The frequency of Wild Boar-derived *IGF2* alleles was 59% in contrast to the expected 50% and there was twice as many "Wild Boar" as "Large White" homozygotes. This deviation was observed with all three loci at the distal tip and is thus not due to typing errors. The effect was also observed with other loci but the degree of distortion decreased as a function of the distance to the distal tip of the chromosome. Blood samples for DNA preparation were collected at 12 weeks of age and we are convinced that the deviation from expected Mendelian ratios was present at birth as the number of animals lost prior to blood sampling was not sufficient to cause a deviation of this magnitude. No other of the more than 250 loci analyzed in this pedigree show such a marked segregation distortion (L. Andersson, unpublished). The segregation distortion did not show an imprinting effect, as the frequencies of the two reciprocal types of heterozygotes were identical (Table 1). This does not exclude the possibility that the QTL effects and the segregation distortion are controlled by the same locus. The segregation distortion maybe due to meiotic drive favoring the paternally expressed allele during gametogenesis, as the  $F_1$  parents were all sired by Wild Boar males. Another possibility is that the segregation distortion may be due to codominant expression of the maternal and paternal allele in some tissues and/or during a critical period of embryo development. Biallelic *IGF2* expression has been reported to occur to some extent



during human development<sup>10, 11</sup> and interestingly a strong influence of the parental species background on *IGF2* expression was recently found in a cross between *Mus musculus* and *Mus spretus*<sup>12</sup>. It is also interesting that a VNTR polymorphism at the insulin gene, which is very closely linked to *IGF2*, is associated with size at birth in humans<sup>13</sup>. It is possible that the *IGF2*-linked QTL in pigs has a minor effect on birth weight but in our data it was far from significant (Fig. 2) and there was no indication of an imprinting effect.

This study is an advance in the general knowledge concerning the biological importance of the *IGF2* locus. The important role of *IGF2* for prenatal development is well-documented from knock-out mice<sup>14</sup> as well as from its causative role in the human Beckwith-Wiedemann syndrome<sup>15</sup>. This study demonstrates an important role for the *IGF2*-region also for postnatal development. It should be stressed that our intercross between outbred populations is particularly powerful to detect QTL with a parent of origin-specific effect on a multifactorial trait. This is because multiple alleles (or haplotypes) are segregating and we could deduce whether a heterozygous F<sub>2</sub> animal received the Wild Boar allele from the F<sub>1</sub> male or female. It is quite possible that the segregation of a paternally expressed *IGF2*-linked QTL affecting a trait like obesity has been overlooked in human studies or in intercrosses between inbred rodent populations because of experimental design or statistical treatment of data. An imprinting effect cannot be detected in an intercross between two inbred lines as only two alleles are segregating at each locus. Our result has therefore significant bearings on the future analysis of the association between genetic polymorphism in the *insulin-IGF2* region and Type I diabetes<sup>16</sup>, obesity<sup>17</sup>, and variation in birth weight<sup>13</sup> in humans, as

well as for the genetic dissection of complex traits using inbred rodent models. A major impetus for generating an intercross between the domestic pig and its wild ancestor was to explore the possibilities to map and identify major loci that have responded to selection. We have now showed that two single QTLs on chromosome 2 (this study) and 4<sup>1</sup>, 2 explain as much as one third of the phenotypic variance for lean meat content in the F<sub>2</sub> generation. This is a gross deviation from the underlying assumption in the classical infinitesimal model in quantitative genetics theory namely that quantitative traits are controlled by an infinite number of loci each with an infinitesimal effect. If a large proportion of the genetic difference between two divergent populations (e.g. Wild Boar and Large White) is controlled by a few loci, one would assume that selection would quickly fix QTL alleles with large effects leading to a selection plateau. However, this is not the experience in animal breeding programs or selection experiments where good persistent long-term selection responses are generally obtained, provided that the effective population size is reasonably large<sup>18</sup>. A possible explanation for this paradox is that QTL alleles controlling a large proportion of genetic differences between two populations may be due to several consecutive mutations; this may be mutations in the same gene or at several closely linked genes affecting the same trait. It has been argued that new mutations contribute substantially to long-term selection responses<sup>19</sup>, but the genomic distribution of such mutations are unknown.

The search for a single causative mutation is the paradigm as regards the analysis of genetic defects in mice and monogenic disorders in humans. We propose that this may not be the case for loci that have been under selection for a large number of generations in domestic animals, crops, or natural populations. This hypothesis

predicts the presence of multiple alleles at major QTL. It gains some support from our recent characterization of porcine coat color variation. We have found that both the alleles for dominant white color and for black-spotting differ from the corresponding wild-type alleles by at least two consecutive mutations with phenotypic effects at the *KIT* and *MC1R* loci, respectively<sup>20, 21</sup>. In this context it is highly interesting that in the accompanying example we have identified a third allele at the *IGF2*-linked QTL. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found i.e. Piétrain pigs are more muscular than Large White pigs that in turn have higher lean meat content than Wild Boars.

There are good reasons to decide that *IGF2* is the causative gene for the now reported QTL. Firstly, there is a perfect agreement in map localization (Fig. 2). Secondly, it has been shown that *IGF2* is paternally expressed in mice, humans, and now in pigs, like the QTL. There are several other imprinted genes in the near vicinity of *IGF2* in mice and humans (*Mash2*, *INS2*, *H19*, *KVLQT1*, *TAPA1/CD81*, and *CDKN1C/p57<sup>KIP2</sup>*) but only *IGF2* is paternally expressed in adult tissues<sup>22</sup>. We believe that this locus provides a unique opportunity for molecular characterization of a QTL. The clear paternal expression can be used to exclude genes that do not show this mode of inheritance. Moreover, the presence of an allelic series should facilitate the difficult distinction between causative mutations and linked neutral polymorphism. We have already shown that there is no difference in coding sequence between *IGF2* alleles from Piétrain and Large White pigs suggesting that the causative mutations occur in regulatory sequences. An obvious step is to sequence the entire *IGF2* gene and its multiple promoters from the three populations. The recent

report that a VNTR polymorphism in the promoter region of the insulin (*INS*) gene affects *IGF2* expression<sup>23</sup> suggests that the causative mutations may be at a considerable distance from the *IGF2* coding sequence.

- 5           The results have several important implications for the pig breeding industry. They show that genetic imprinting is not an esoteric academic question but need to be considered in practical breeding programs. The detection of three different alleles in Wild Boar, Large
- 10   White, and Piétrain populations indicates that further alleles at the *IGF2*-linked QTL segregate within commercial populations. The paternal expression of the QTL facilitates its detection using large paternal half-sib families as the female contribution can be ignored.
- 15   The QTL is exploited to improve lean meat content by marker assisted selection within populations or by marker assisted introgression of favorable alleles from one population to another.

## Example 2: Piétrain x Large White intercrosses

## Methods

- Pedigree material:* The pedigree material utilized to map
- 5 QTL was selected from a previously described Piétrain x Large White F2 pedigree comprising > 1,800 individuals<sup>6,7</sup>. To assemble this F2 material, 27 Piétrain boars were mated to 20 Large White sows to generate an F1 generation comprising 456 individuals. 31 F1 boars were mated to
- 10 unrelated 82 F1 sows from 1984 to 1989, yielding a total of 1862 F2 offspring. F1 boars were mated on average to 7 females, and F1 sows to an average of 2,7 males. Average offspring per boar were 60 and per sow 23.
- 15 *Phenotypic information: (i) Data collection:* A total of 21 distinct phenotypes were recorded in the F2 generation<sup>6,7</sup>. These included:
- five growth traits: birth weight (g), weaning weight (Kg), grower weight (Kg), finisher weight (Kg) and
  - 20 average daily gain (ADG; Kg/day; grower to finisher period);
  - two body proportion measurements: carcass length (cm); and a conformation score (0 to 10 scale; ref.6);
  - ten measurements of carcass composition obtained by
  - 25 dissection of the chilled carcasses 24 hours after slaughter. These include measurements of muscularity: % ham (weight hams/carcass weight), % loin (weight loin/carcass weight), % shoulder (weight
  - 30 shoulder/carcass weight), % lean cuts (% ham + %loin + % shoulder); and measurements of fatness: average back-fat thickness (BFT; cm), % backfat (weight backfat/carcass weight), % belly (weight belly/carcass weight), % leaf fat (weight leaf fat/carcass weight), % jowl (weight jowl/carcass weight), and "% fat cuts" (% backfat + %
  - 35 belly + % leaft fat + % jowl).
  - four meat quality measurements: pH<sub>LD1</sub> (*Longissimus dorsi* 1

hour after slaughter), pH<sub>LD24</sub> (*Longissimus dorsi* 24 hours after slaughter), pH<sub>G1</sub> (*Gracilis* 1 hour after slaughter) and pH<sub>G24</sub> (*Gracilis* 24 hours after slaughter). (ii) *Data processing*: Individual phenotypes were preadjusted for fixed effects (sire, dam, CRC genotype, sex, year-season, parity) and covariates (litter size, birth weight, weaning weight, grower weight, finisher weight) that proved to significantly affect the corresponding trait. Variables included in the model were selected by stepwise regression.

10

*Marker genotyping*: Primer pairs utilized for PCR amplification of microsatellite markers are as described<sup>19</sup>. Marker genotyping was performed as previously described<sup>20</sup>. Genotypes at the *CRC* and *MyoD* loci were determined using conventional methods as described<sup>1,12</sup>. The LAR test for the *Igf2* SNP was developed according to Baron et al.<sup>21</sup> using a primer pair for PCR amplification (5'-CCCCTGAACTTGAGGACGAGCAGCC-3'; 5'-ATCGCTGTGGGCTGGGTGGGCTGCC-3') and a set of three primers for the LAR step (5'-FAM-CGCCCCAGCTGCCCCCAG-3'; 5'-HEX-CGCCCCAGCTGCCCCCAA-3'; 5'-CCTGAGCTGCAGCAGGCCAG-3').

20

*Map construction*: Marker maps were constructed using the TWOPOINT, BUILD and CHROMPIC options of the CRIMAP package<sup>22</sup>. To allow utilisation of this package, full-sib families related via the boar or sow were disconnected and treated independently. By doing so, some potentially usable information was neglected, yielding, however, unbiased estimates of recombination rates.

25

*QTL mapping*: (i) *Mapping Mendelian QTL*: Conventional QTL mapping was performed using a multipoint maximum likelihood method. The applied model assumed one segregating QTL per

30

chromosome, and fixation of alternate QTL alleles in the respective parental lines, Piétrain (P) and Large White (LW). A specific analysis program had to be developed to account for the missing genotypes of the parental generation, resulting in the fact that the parental origin of the F1 chromosomes could not be determined. Using a typical "interval mapping" strategy, an hypothetical QTL was moved along the marker map using user-defined steps. At each position, the likelihood ( $L$ ) of the pedigree data was computed as:

$$L = \sum_{\varphi=1}^{2^r} \prod_{i=1}^n \sum_{G=1}^4 (P(G|M_i, \theta, \varphi) P(y_i|G))$$

$P$  or right chromosome  $P$ ), there is a total of  $2^r$  combinations for  $r$  F1 parents.

15  $\prod_{i=1}^n$  n F2

$\sum_{G=1}^4$  ith F2 offspring, over the four possible QTL genotypes:

$P/P$ ,  $P/LW$ ,  $LW/P$  and  $LW/LW$

$P(G|M_i, \theta, \varphi)$   $M_i$ : the marker genotype of the  $i$ th F2 offspring and its F1 parents, (ii) : the vector of recombination rates between adjacent markers and between the hypothetical QTL and its flanking markers, and (iii)  $\theta$  the considered marker-QTL phase combination of the F1 parents.

Recombination rates and marker linkage phase of F1 parents are assumed to be known when computing this probability. Both were determined using CRIMAP in the map construction phase (see above).

$P(y_i|G)$   $y_i$ ) of offspring  $i$ , given the QTL genotype under consideration. This probability is computed from the normal density function:

$$P(y_i|G) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(y_i - \mu_g)^2}{2\sigma^2}}$$

$\mu_g$  is the phenotypic mean of the considered QTL genotype (PP, PL, LP or LL) and  $\sigma^2$  the residual variance  $\sigma^2$  was considered to be the same for the four QTL genotypic classes.

- 5 The values of  $\mu_{PP}$ ,  $\mu_{PL}=\mu_{LP}$ ,  $\mu_{LL}$  and  $\sigma^2$  maximizing  $L$  were determined using the GEMINI optimisation routine<sup>23</sup>.

The likelihood obtained under this alternative  $H_1$  hypothesis was compared with the likelihood obtained under the null hypothesis  $H_0$  of no QTL, in which the phenotypic means of the

- 10 four QTL genotypic classes were forced to be identical. The difference between the logarithms of the corresponding likelihoods yields a lodscore measuring the evidence in favour of a QTL at the corresponding map position.

(ii) *Significance thresholds:* Following Lander & Botstein<sup>24</sup>,

- 15 lodscore thresholds ( $T$ ) associated with a chosen genome-wide significance level, were computed such that:

$$\alpha = (C + 9.21GT)\chi_2^2(4.6T)$$

$C$  corresponds to the number of chromosomes (= 19),  $G$

corresponds to the length of the genome in Morgans (= 29),

- 20 and  $\chi_2^2(4.6T)$  denotes one minus the cumulative distribution function of the chi-squared distribution with 2 d.f. Single point  $2\ln(LR)$  were assumed to be distributed as a chi-squared distribution with two degrees of freedom, as we were fitting both an additive and dominance component. To account for the
- 25 fact that we were analysing multiple traits, significance levels were adjusted by applying a Bonferoni correction corresponding to the effective number of independent traits that were analyzed. This effective number was estimated at 16 following the approach described by Spelman et al.<sup>25</sup>.

- 30 Altogether, this allowed us to set the lodscore threshold associated with an experiment-wise significance level of 5%



at 5.8. When attempting to confirm the identified QTL in an independent sample, the same approach was used, however, setting C at 1, G at 25cM and correcting for the analysis of 4.5 independent traits (as only six traits were analyzed in this sample). This yielded a lodscore threshold associated with a Type I error of 5% of 2.

(iii). *Testing for an imprinted QTL*: To test for an imprinted QTL, we assumed that only the QTL alleles transmitted by the parent of a given sex would have an effect on phenotype, the QTL alleles transmitted by the other parent being "neutral". The likelihood of the pedigree data under this hypothesis was computed using equation 1. To compute  $P(y_i | G)$ , however, the phenotypic means of the four QTL genotypes were set at  $\mu_{PP} = \mu_{PL} = \mu_P$  and  $\mu_{LP} = \mu_{LL} = \mu_L$  to test for a QTL for which the paternal allele only is expressed, and  $\mu_{PP} = \mu_{LP} = \mu_P$  and  $\mu_{PL} = \mu_{LL} = \mu_L$  to test for a QTL for which the maternal allele only is expressed. It is assumed in this notation that the first subscript refers to the paternal allele, the second subscript to the maternal allele.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  testing the presence of a Mendelian QTL;  $H_2$  testing the presence of a paternally expressed QTL, and  $H_3$  testing the presence of a maternally expressed QTL.

*RT-PCR*: Total RNA was extracted from skeletal muscle according to Chirgwin et al.<sup>26</sup>. RT-PCR was performed using the Gene-Amp RNA PCR Kit (Perkin-Elmer) The PCR products were purified using QiaQuick PCR Purification kit (Qiagen) and sequenced using Dye terminator Cycle Sequencing Ready Reaction (Perkin Elmer) and an ABI373 automatic sequencer.

In example 2 we report the identification of a QTL with major effect on muscle mass and fat deposition mapping to porcine 2p1.7. The QTL shows clear evidence for parental imprinting strongly suggesting the involvement of the *Igf2* locus.

5        A Piétrain X Large White intercross comprising 1125 F<sub>2</sub> offspring was generated as described<sup>6,7</sup>. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famed for their exceptional muscularity and leanness<sup>8</sup> (Figure 2), while Large  
10        Whites show superior growth performance. Twenty-one distinct phenotypes measuring (i) growth performance (5), (ii) muscularity (6), (iii) fat deposition (6), and (iv) meat quality (4), were recorded on all F<sub>2</sub> offspring.

         In order to map QTL underlying the genetic differences  
15        between these breeds, we undertook a whole genome scan using microsatellite markers on an initial sample of 677 F<sub>2</sub> individuals. Analysis of pig chromosome 2 using a ML multipoint algorithm, revealed highly significant lodscores (up to 20) for six of the 12 phenotypes measuring muscularity  
20        and fat deposition at the distal end of the short arm of chromosome 2 (Figure 3a). Positive lodscores were obtained for the remaining six phenotypes, however, not reaching the genome-wide significance threshold ( $\alpha = 5\%$ ). To confirm this finding, the remaining sample of 355 F<sub>2</sub> offspring was  
25        genotyped for the five most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for  
30        the three genotypic means as well as the corresponding residual variance.

         Bidirectional chromosome painting establishes a correspondence between SSC2p and HSA11pter-q13<sup>9,10</sup>. At least

two serious candidate genes map to this region in man: the myogenic basic helix-loop-helix factor, *MyoD*, maps to HSA11p15.4, while *Igf2* maps to HSA11p15.5. *MyoD* is a well known key regulator of myogenesis and is one of the first myogenic markers to be switched on during development<sup>11</sup>. A previously described amplified sequence polymorphism in the porcine *MyoD* gene<sup>12</sup> proved to segregate in our F<sub>2</sub> material, which was entirely genotyped for this marker. Linkage analysis positioned the *MyoD* gene in the SW240-SW776 (odds > 1000) interval, therefore well outside the lod-2 drop off support interval for the QTL (figure 1). *Igf2* is known to enhance both proliferation and differentiation of myoblasts *in vitro*<sup>13</sup> and to cause a muscular hypertrophy when overexpressed *in vivo*. Based on a published porcine adult liver cDNA sequence<sup>14</sup>, we designed primer pairs allowing us to amplify the entire *Igf2* coding sequence with 222 bp of leader and 280 bp of trailer sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced indicating that the coding sequences was identical in both breeds and with the published sequence. However, a G A transition was found in the leader sequence corresponding to exon 2 in man (Figure 4). We developed a screening test for this single nucleotide polymorphism (SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, *Igf2* was shown to colocalize with the SWC9 microsatellite marker (= 0%), therefore located at approximately 2 centimorgan from the most likely position of the QTL and well within the 95% support interval for the QTL (figure 1). Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3' UTR of the *Igf2* gene. Combined with available comparative mapping data for the PGA and FSH loci, these results suggest the occurrence of an interstitial

inversion of a chromosome segment containing *MyoD*, but not *Igf2* which has remained telomeric in both species.

*Igf2* therefore appeared as a strong positional allele having the observed QTL effect. In man and mouse, *Igf2* is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues<sup>15</sup>. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9, shows that the same imprinting holds in this tissue in the pig as well (Figure 4). Therefore if *Igf2* were responsible for the observed effect, and knowing that only the paternal *Igf2* allele is expressed, one can predict that (i) the paternal allele transmitted by F1 boars (P or LW) would have an effect on phenotype of F2 offspring, (ii) the maternal allele transmitted by F1 sows (P or LW) would have no effect on phenotype of F2 offspring, and (iii) the likelihood of the data would be superior under a model of a bimodal (1:1) F2 population sorted by inherited paternal allele when compared to a conventional "Mendelian" model of a trimodal (1:2:1) F2 population. The QTL mapping programs were adapted in order to allow testing of the corresponding hypotheses.  $H_0$  was defined as the null-hypothesis of no QTL,  $H_1$  as testing for the presence of a Mendelian QTL,  $H_2$  as testing for the presence of a paternally expressed QTL, and  $H_3$  as testing for the presence of a maternally expressed QTL.

Figure 3 summarizes the obtained results. Figure 3a, 3b and 3c respectively show the lodscore curves corresponding to  $\log_{10} (H_2/H_0)$ ,  $\log_{10} (H_3/H_0)$  and  $\log_{10} (H_2/H_1)$ . It can be seen that very significant lodscores are obtained when testing for the presence of a paternally expressed QTL, while there is no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. Also, the hypothesis of a paternally expressed QTL is significantly more likely ( $\log_{10} (H_2/H_1) > 3$ ) than the hypothesis of a "Mendelian" QTL

for all examined traits. The fact that the same tendency is observed for all traits indicates that it is likely the same imprinted gene that is responsible for the effects observed on the different traits. Table 2 reports the ML phenotypic means for the F2 offspring sorted by inherited paternal QTL allele. Note that when performing the analysis under a model of a mendelian QTL, the Piétrain and Large White QTL alleles appeared to behave in an additive fashion, the heterozygous genotype exhibiting a phenotypic mean corresponding exactly to the midpoint between the two homzygous genotypes. This is exactly what one would predict when dealing with an imprinted QTL as halve of the heterozygous offspring are expected to have inherited the P allele from their sire, the other halve the LW allele.

These data therefore confirmed our hypothesis of the involvement of an imprinted gene expressed exclusively from the paternal allele. The fact that the identified chromosomal segment coincides precisely with an imprinted domain documented in man and mice strongly implicates the orthologous region in pigs. At least seven imprinted genes mapping to this domain have been documented (*Igf2*, *Ins2*, *H19*, *Mash2*, *p57<sup>KIP2</sup>*, *K<sub>v</sub>LQTL1* and *TDAG51*) (ref. 15 and Andrew Feinberg, personal communication). Amongst these, only *Igf2* and *Ins2* are paternally expressed. While we cannot exclude that the observed QTL effect is due to an as of yet unidentified imprinted gene in this region, its reported effects on myogenesis *in vitro* and *in vivo*<sup>13</sup> strongly implicate *Igf2*. Particularly the muscular hypertrophy observed in transgenic mice overexpressing *Igf2* from a muscle specific promotor are in support of this hypothesis (Nadia Rosenthal, personal communication. Note that allelic variants of the *INS* VNTR have recently been shown to be associated

with size at birth in man<sup>16</sup>, and that the same VNTR has been shown to affect the level of *Igf2* expression<sup>17</sup>.

The observation of the same QTL effect in a Large White x Wild Boar intercross indicates the existence of a series of  
5 at least three distinct functional alleles. Moreover, preliminary evidence based on marker assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series might be invaluable in  
10 identifying the causal polymorphisms which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations.

The effects of the identified QTL on muscle mass and fat  
15 deposition are truly major, being of the same magnitude of those reported for the CRC locus<sup>6,7</sup> though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity  
20 and leanness. Understanding the parent-of-origin effect characterizing this locus will allow for its optimal use in breeding programs. Indeed, today half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing  
25 considerable loss.

The QTL described in this work is the second example of a gene affecting muscle development in livestock species that exhibits a non-mendelian inheritance pattern. Indeed, we have previously shown that the callipyge locus (related to the  
30 qualitative trait wherein muscles are doubled) is characterized by polar overdominance in which only the heterozygous individuals that inherit the CLPG mutation from their sire express the double-muscling phenotype<sup>5</sup>. This

demonstrates that parent-of-origin effects affecting genes underlying production traits in livestock might be relatively common.

5 Example 3:

Generating a reference sequence of IGF2 and flanking loci in the pig.

- 10 The invention provides an imprinted QTL with major effect on muscle mass mapping to the IGF2 locus in the pig, and use of the QTL as tool in marker assisted selection. To fine tune this tool for marker assisted selection, as well as to further identify a causal mutation, we have further generated  
15 a reference sequence encompassing the entire porcine IGF2 sequence as well as that from flanking genes.

- To achieve this, we screened a porcine BAC library with IGF2 probes and identified two BACs. BAC-PIGF2-1 proved to  
20 contain the INS and IGF2 genes, while BAC-PIGF2-2 proved to contain the IGF2 and H19 genes. The NotI map as well as the relative position of the two BACs is shown in Figure 5. BAC-PIGF2-1 was shotgun sequenced using standard procedures and automatic sequencers. The resulting sequences were assembled  
25 using standard software yielding a total of 115 contigs. The corresponding sequences are reported in figure 6. Similarity searches were performed between the porcine contigs and the orthologous sequences in human. Significant homologies were detected for 18 contigs and are reported in Figure 7.

30

For BAC-PIGF2-2, the 24 Kb NotI fragment not present in BAC-PIGF2-1 was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers. Resulting

sequences were assembled using the Phred-Phrap-Consed program suit, yielding seven distinct contigs (figure 8). The contig sequences were aligned with the corresponding orthologous human sequences using the compare and dotplot programs of the GCG suite. Figure 9 summarizes the corresponding results.

Example 4: Identification of DNA sequence polymorphisms in the IGF2 and flanking loci.

10 Based on the reference sequence obtained as described in Example 1, we resequenced part of the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals, allowing identification of DNA sequence polymorphisms such as reported in figure 10.

15



## Legends to the figures

Fig. 1: Test statistic curves obtained in QTL analyses of  
5 chromosome 2 in a Wild Boar/Large White intercross. The graph  
plots the F ratio testing the hypothesis of a single QTL at a  
given position along the chromosome for the traits indicated.  
The marker map with the distances between markers in Kosambi  
10 centiMorgan is given on the X-axis. The horizontal lines  
represent genome-wise significant ( $P < 0.05$ ) and suggestive  
levels for the trait lean meat in ham; similar significance  
thresholds were obtained for the other traits.

Figure 2: Piétrain pig with characteristic muscular  
15 hypertrophy.

Figure 3: Lodscore curves obtained in a Piétrain x Large  
White intercross for six phenotypes measuring muscle mass and  
fat deposition on pig chromosome 2. The most likely positions  
20 of the *Igf2* and *MyoD* genes determined by linkage analysis  
with respect to the microsatellite marker map are shown.  $H_0$   
was defined as the null-hypothesis of no QTL,  $H_1$  as testing  
for the presence of a Mendelian QTL,  $H_2$  as testing for the  
presence of a paternally expressed QTL, and  $H_3$  as testing for  
25 the presence of a maternally expressed QTL. 3a:  $\log_{10}(H_1/H_0)$ ,  
3b:  $\log_{10}(H_2/H_0)$ , 3c:  $\log_{10}(H_3/H_0)$

Figure 4: A. Structure of the human *Igf2* gene according to  
ref. 17, with aligned porcine adult liver cDNA sequence as  
30 reported in ref. 16. The position of the nt241(G-A)  
transition and Swc9 microsatellite are shown. B. The  
corresponding markers were used to demonstrate the  
monoallelic (paternal) expression of *Igf2* in skeletal muscle

and liver of 10-week old fetuses. PCR amplification of the *nt421(G-A)* polymorphism and *Swc9* microsatellite from genomic DNA clearly shows the heterozygosity of the fetus, while only the paternal allele is detected in liver cDNA (*nt421(G-A)* and *Swc9*) and muscle cDNA (*Swc9*). The absence of RT-PCR product for *nt421(G-A)* from in fetal muscle points towards the absence of mRNA including exon 2 in this tissue. Parental origin of the foetal alleles was determined from the genotypes of sire and dam (data not shown).

10

Figure 5: A NotI restriction map showing the relative position of BAC-PIGF2-1 (comprising INS and IGF2 genes), and BAC-PIGF2-2 (comprising IGF2 and H19 genes).

15 Figure 6: Nucleic acid sequences of contig 1 to contig 115 derived from BAC-PIGF2-1 which was shotgun sequenced using standard procedures and automatic sequencers.

Figure 7: Similarity between porcine contigs of figure 6 and orthologous sequences in human.

20

Figure 8 Nucleic acid sequences of contig 1 to contig 7 derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1) which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic sequencers.

25

Figure 9: Similarity between porcine contigs of figure 8 and orthologous sequences in human.

30

Figure 10: DNA sequence polymorphisms in the IGF2 and flanking loci from genomic DNA isolated from Piétrain, Large White and Wild Boar individuals.

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Table 1 Summary of QTL analysis for pig chromosome 2 in a Wild Boar/Large White intercross<sup>1</sup>

Trait	F ratio <sup>2</sup>	Imprinting	Map position <sup>3</sup>	Percent of F <sub>2</sub> variance <sup>4</sup>	Least squares means <sup>5</sup>			
	QTL				wP/wM	wP/LM	L <sup>P</sup> /wM	
<u>Body composition traits</u>								
5	L <sup>P</sup> /LM							
					n=62	n=43	n=30	
	Lean meat in ham, %	24.4***	19.1***	0	30.6	63.6 <sup>a</sup>	64.2 <sup>a</sup>	66.4 <sup>b</sup>
	Lean meat mass in ham, kg	18.1***	16.8***	1	24.3	4.69 <sup>a</sup>	4.72 <sup>a</sup>	4.94 <sup>b</sup>
10	Lean meat + bone in back, %	12.2**	9.6**	0	17.4	66.3 <sup>a</sup>	66.7 <sup>a</sup>	69.3 <sup>b</sup>
	Longissimus muscle area, cm <sup>2</sup>	10.3**	4.8*	1	15.4	31.9 <sup>a</sup>	33.0 <sup>a</sup>	34.5 <sup>b</sup>
<u>Fatness traits</u>								
15	Average back fat depth, mm	7.1*	8.7**	0	10.4	27.2 <sup>a</sup>	27.7 <sup>a</sup>	25.5 <sup>b</sup>
<u>Weight of internal organs</u>								
	Heart, gram	9.7**	11.4***	0	14.4	226 <sup>a</sup>	225 <sup>a</sup>	238 <sup>b</sup>
<u>Meat quality traits</u>								
20	Reflectance value, EEL	5.7	6.1*	1	8.1	18.6 <sup>a</sup>	18.4 <sup>a</sup>	21.8 <sup>b</sup>

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001

\*P&lt;0.05; \*\*P&lt;0.01; \*\*\*P&lt;0.001

**Table 1, continued**

- <sup>1</sup>Only the traits for which the QTL peak was in the *IGF2* region (0-10 cM) and the test statistic reached the nominal significance threshold of  $F=3.9$  are included.
- <sup>2</sup>"QTL" is the test statistic for the presence of a QTL under a genetic model with additive, dominance, and imprinting effects (3 d.f.) while "Imprinting" is the test statistic for the presence of an imprinting effect (1 d.f.), both obtained at the position of the QTL peak. Genome-wide significance thresholds, estimated by permutation, were used for the QTL test while nominal significance thresholds were used for the Imprinting test.
- <sup>3</sup>In cM from the distal end of 2p; *IGF2* is located at 0.3 cM.
- <sup>4</sup>The reduction in the residual variance of the  $F_2$  population effected by inclusion of an imprinted QTL at the given position.
- <sup>5</sup>Means and standard errors estimated at the *IGF2* locus by classifying the genotypes according to the population and parent of origin of each allele. *W* and *L* represent alleles derived from the Wild Boar and Large White founders, respectively; superscript *P* and *M* represent a paternal and maternal origin, respectively. Figures with different letters (superscript a or b) are significantly different at least at the 5% level, most of them are different at the 1% or 0.1% level.

Table 2 Maximum likelihood phenotypic means for the different F2 genotypes estimated under (i) a model of a mendelian QTL, and (ii) a model assuming an imprinted QTL.

Traits	Mendelian QTL				Imprinted QTL		
	$\mu_{LW/LW}$	$\mu_{LW/P}$	$\mu_{P/P}$	R	$\mu_{PAT/LW}$	$\mu_{PAT/P}$	R
BFT (cm)	2.98	2.84	2.64	0.27	2.94	2.70	0.27
% ham	21.10	21.56	22.15	0.83	21.23	21.9 5	0.83
% loin	24.96	25.53	26.46	0.91	25.12	26.1 4	0.93
% lean cuts	65.02	65.96	67.60	1.65	65.23	67.0 5	1.67
% backfat	6.56	6.02	5.33	0.85	6.43	5.56	0.85
% fat cuts	28.92	27.68	26.66	1.46	28.54	26.9 9	1.49

CLAIMS

1. A method for selecting a domestic animal for having desired genotypic properties comprising testing said animal for the presence of a parentally imprinted quantitative trait locus (QTL).
- 5 2. A method according to claim 1 further comprising testing a nucleic acid sample from said animal for the presence of a parentally imprinted quantitative trait locus (QTL).
3. A method according to claim 1 or 2 wherein in the pig said QTL is located at chromosome 2.
- 10 4. A method according to claim 2 or 3 wherein said QTL is mapping at around position 2p1.7.
5. A method according to claim 1 to 4 wherein said QTL is related to the potential muscle mass and/or fat deposition of said animal.
- 15 6. A method according to claim 5 wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) gene.
7. A method according to anyone of claims 1 to 6 wherein in the pig said QTL comprises a marker characterised as nt241(G-
- 20 A) or as Swc9, as identified in figure 4.
8. A method according to anyone of claims 1-7 wherein a paternal allele of said QTL is predominantly expressed in said animal.
9. A method according to anyone of claims 1-7 wherein a
- 25 maternal allele of said QTL is predominantly expressed in said animal.
10. An isolated and/or recombinant nucleic acid comprising a parentally imprinted quantitative trait locus (QTL) or functional fragment derived thereof.
- 30 11. An isolated and/or recombinant nucleic acid comprising a synthetic parentally imprinted quantitative trait locus (QTL)

derived from at least one chromosome or functional fragment derived thereof.

12. A nucleic acid according to claim 10 or 11 at least partly derived from a *Sus scrofa* chromosome.

5 13. A nucleic acid according to claim 12 wherein said nucleic acid is at least partly derived from a *Sus scrofa* chromosome 2, preferably from a region mapping at around position 2p1.7.

14. A nucleic acid according to any one of claims 10 to 13 wherein said QTL is related to the potential muscle mass  
10 and/or fat deposition of said animal.

15. A nucleic acid according to any one of claims 10 to 14 wherein said QTL comprises at least a part of a insulin-like growth factor-2 (IGF2) gene.

16. A nucleic acid according to anyone of claims 10 to 15  
15 wherein a paternal allele of said QTL is capable of being predominantly expressed.

17. A nucleic acid according to anyone of claims 10 to 16 wherein a maternal allele of said QTL is capable of being predominantly expressed.

20 18. Use of a nucleic acid or fragment derived thereof according to claim 10 in a method according to anyone of claims 1-9.

19. Use according to claim 18 to select a breeding animal or animal destined for slaughter for having desired genotypic or  
25 potential phenotypic properties.

20. Use according to claim 19 wherein said properties are related to muscle mass and/or fat deposition.

21. An animal such as pig selected by a use according to claim 18 to 20.

30 22. A animal according to claim 21 characterised in being homozygous for an allele at a paternally imprinted QTL, preferably located at a *Sus scrofa* chromosome 2 mapping at around position 2p1.7.

23. An animal according to claim 21 or 22 wherein said QTL is  
35 related to the potential muscle mass and/or fat deposition of

said pig and/or wherein said QTL comprises at least a part of a insulin-like growth factor-2 (IGF2) allele.

24. A transgenic animal comprising a nucleic acid according to anyone of claims 11 to 16.

5 25. An animal according to anyone of claims 21-24 which is a male.

26. Sperm or an embryo derived from an animal according to anyone of claims 21-25.

27. Use of a sperm or an embryo according to claim 26 in  
10 breeding animals destined for slaughter.

FIGURE 1

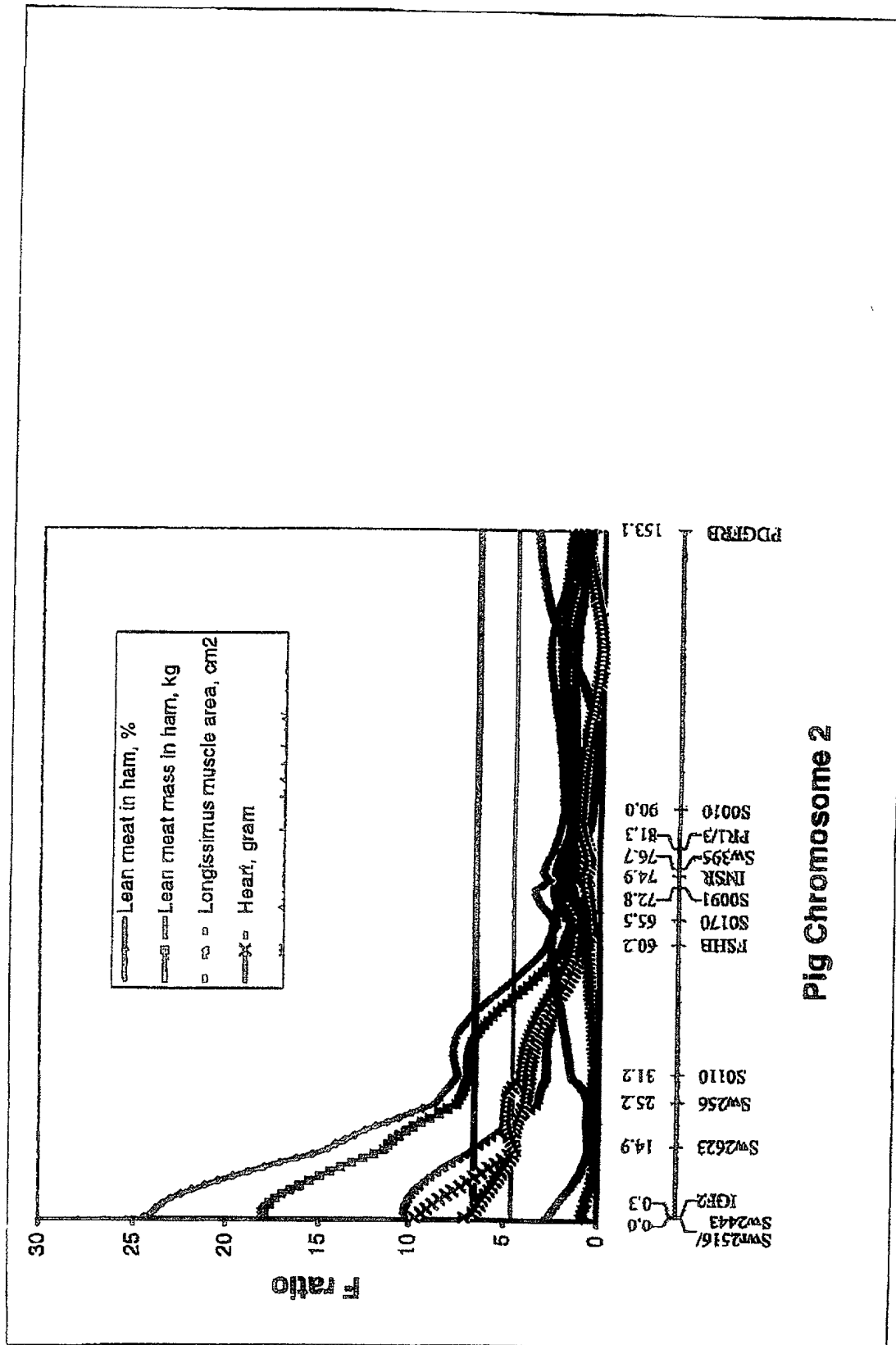


FIGURE 2





FIGURE 3A

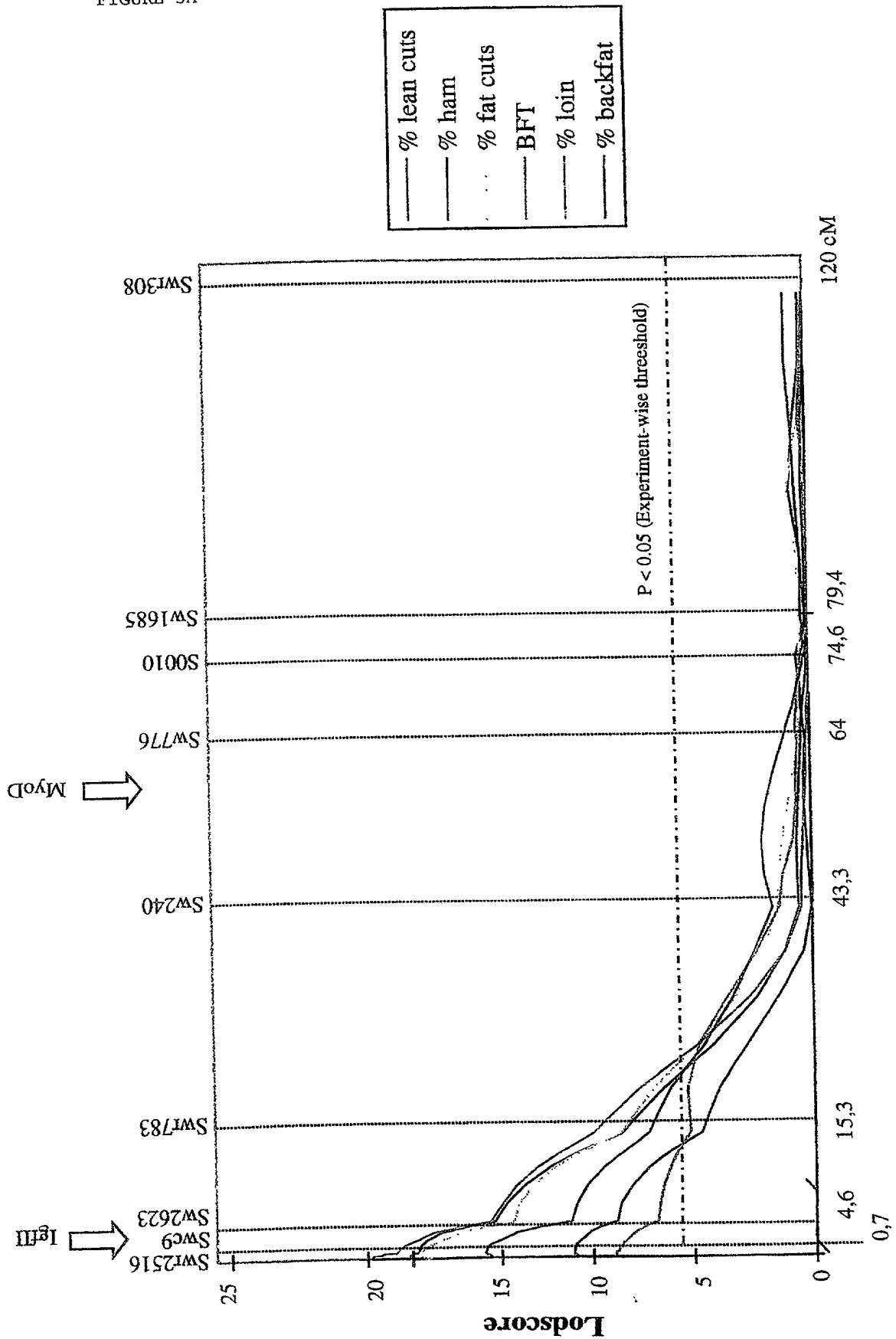


FIGURE 3B

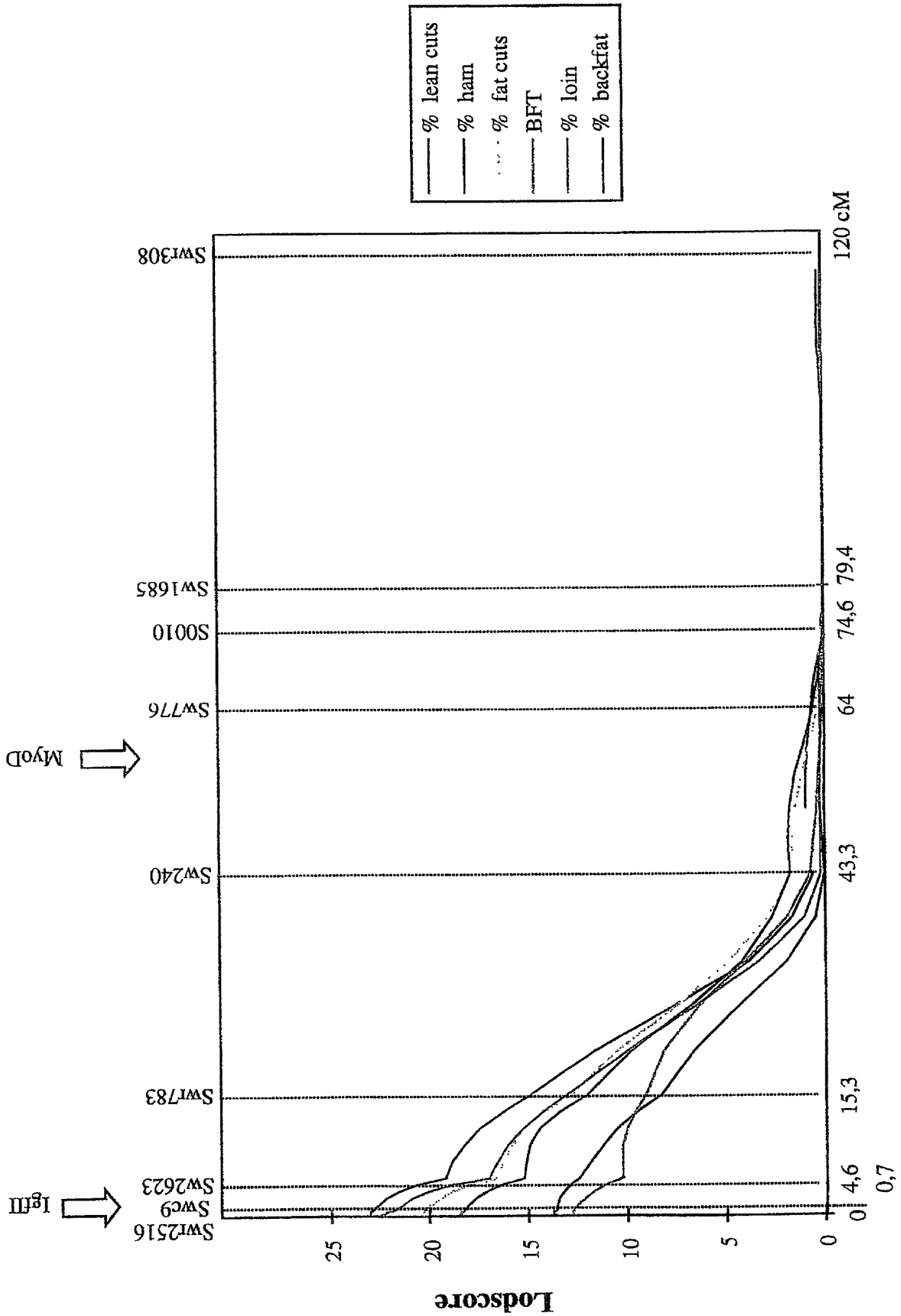


FIGURE 3C

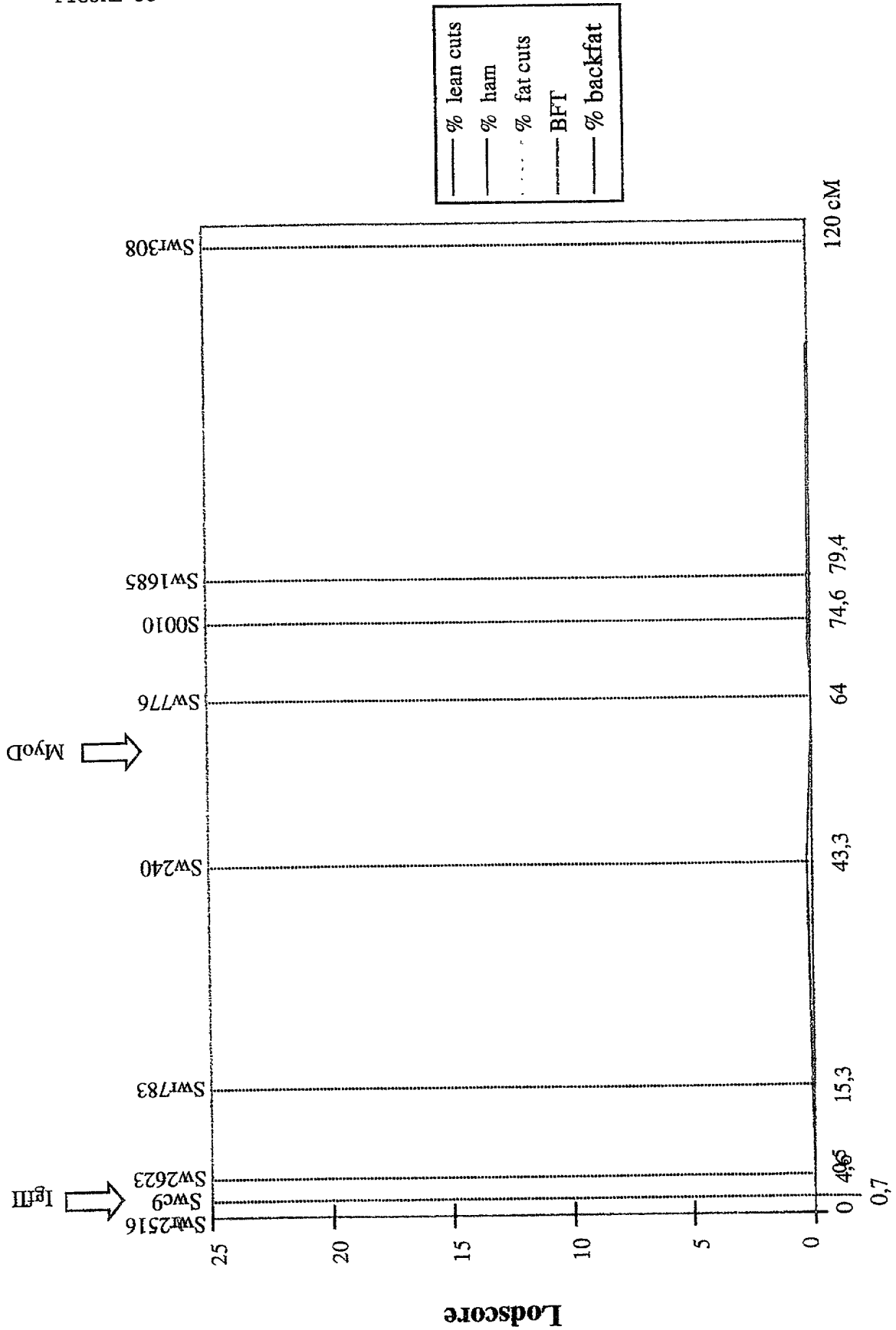


FIGURE 4

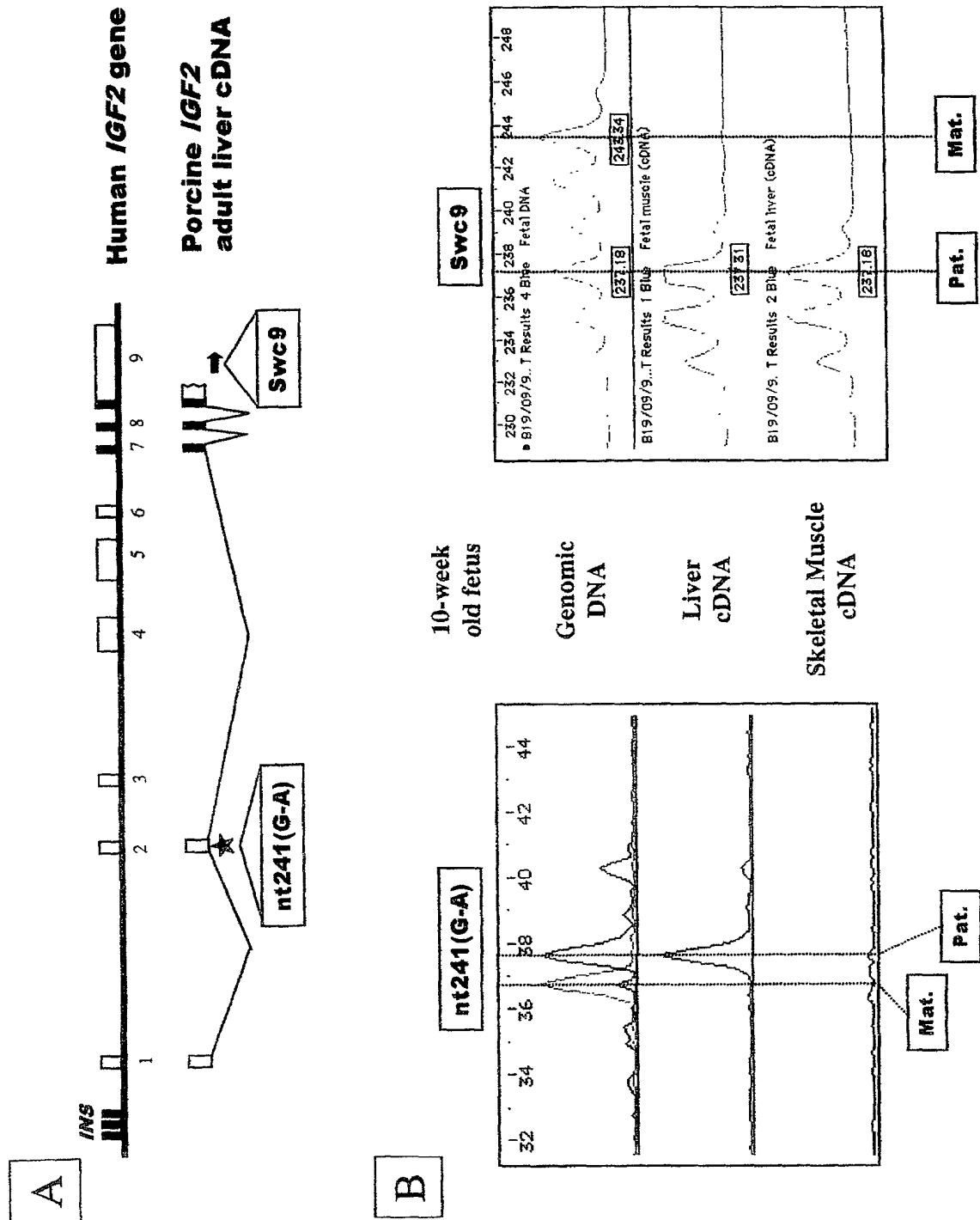


FIGURE 5

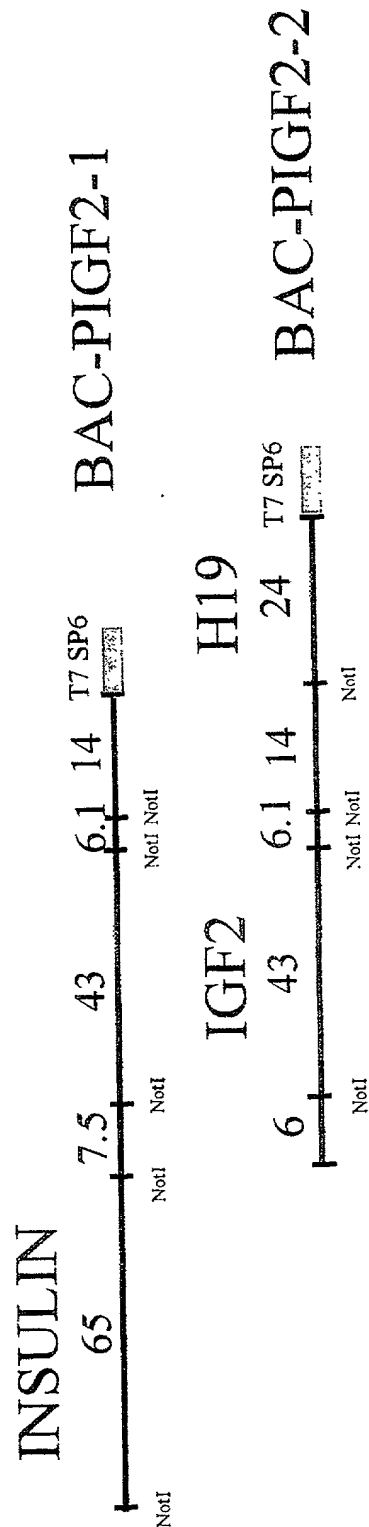


FIGURE 6

## Contig 1 (500 bp)

GGGTGGGCAGCTTCCCTCCAGACCGCAGGAGGCCCAAGTTCCCTGGCCCTGCCACCCAGGGCCAGCTGAAGC  
 AGGTGAGAGACACCCGCTCCTGTCCCTCCTGTACCTAACCCACAGGCCGGGGCCAGGGACACAGGCCACA  
 TGGCATCTCCCCCATGCCCTGCCCAAGGCGCCAGCAGGTGAGGCTGGAGCAGAGTCTGGGTCCCTGCGGG  
 CCAGACCGAGGGCAGGACAGCTGGGCATCTGTCTCACAGTCCCCGCGCTTTGTGCGGGAGGCGGCAGAGCCTC  
 ATCCAAGACGCCCCAAGGAACGGGAGAAGGCGGAGGCGCGGCTGCCGCGTCCGAGCCCGGGGAGGCCCTGG  
 AAGTGGGGGCCCTTGCCGAGCGGACGGGAAGGCCCTGCTGAACCTGCTCTTACCCTGAGGGCCACCAAGCC  
 CCCCTCGCTGTTCCGGTCCCTGAAAAATTCTAGGTGAGGGGGCGGGCCAGGGCTCCCCGGG

## Contig 2 (943 bp)

TGCTCCTCACACCCCGGGCGGGGCTGCTCTTGGGGCCATCCTCCCCATGGGGCCAGCACCCACTCTGGCCTTC  
 ACACCTGCCGTCTTCTGGGAAGTCCTCTGGTTCCCAAGGAAAGTTTCTGAGCTGGACAAGTGCCACCACCTGG  
 TCACCAAGTTTCGATCCTGAGCTGGACCTGGACCACCCGGTGAGCCGGTGCCCTCCCTCCCCGGCCGCATGTC  
 TCCCATCCCCAGGGGTGTCCCCACACTCAGGGCCGGGACTGGGCGTGAACCCCGGGTTGGGACGGATGTTGGC  
 CTGCTGTGTGGCTCCTGGCGGAACAGAGAGGCTGGCTGGGTGCCACCCCGAGGGCCCCCGCCGATGACACGG  
 GCCGCGTCTGGGCTGGGCGGGCAGGGCGGCCAGGC  
 AGGGCAGCCTCCGATGGCTCCCCGGGTGTACCAGGGCTTCTCGGACCAGTTGTACCGCCAGCGCAGGAAGC  
 TGATTGCCCAGATCGCCTTCCAGTACAGGCAGTAAGTCCCTCCAGGGCCTCAGCCTGGGGGCCAGACCTCAG  
 CCTGGGCTCACGCCAGACCTGGGGGTGAGGGAAGGGAGGTTGTCTTTGTACCAACGCCACCACCTTCACT  
 GTACCATGGTACCGACTCTGGGTCCCCAAATCACAGCTGAGGAACTGGGGCACAGAGTGGTTAAGCATCT  
 TGCTGAAGCCACACAGCTGGCGGAGCATTGGCCCCGGCCCTCCTGCGGCTCCACACGTGCTCCCTGAGGG  
 CCCCCGACTGACAGCTGTCCCTCCTCAGAGGTG  
 ACCCTATTCCCGCTGAGTACACAGCCGAGGAGATTGCCACCTGGTGAGGCCCTGTGACAGCGGCTGGGAG  
 GGGCGGAGTGGGGGAAGGGACAGGAAGACCTCAGAATTCCCGCTGGAACGTGGTGGCCTCTATCATGA

## Contig 3 (1500 bp)

GGGGAGGGGATGCTCAGACCCGCTCTGGGAAGAAGAGAGCCTCAGAAGAAATCCCTTCCCAAGGGTCACGCGG  
 TGGAGCCCAGGGGCCCGCTAGGGGCCGATFCCACAGCTCGTGCTGCCACCTGCTGGCGCTCCAGGAACCTGC  
 GGAGCGGTGGGGGCCCTGGATGGGTCCGGCAGTGGGCTCGCAGGAGACCCCTGGAGGGGCTGCGGACACCCC  
 AGCTGCCACTCACAAGGTGCCAAGCGGCGGTGGCAATGGGCTGAGCCTCTCCCCCTCCTCCTCCGAGGA  
 CATTGGCCTCGCATCCCTGGGGGTCTCGGACGAGGAAATTGAGAAGCTGTCCACGCTGGGTTTCTCCCCCTGC  
 AGGGCCCTGGGTTCCAGCCAGGCCCTCCTGTCCAA  
 GGGGTGTCGTCTCAGCTGTGACCGCCCGGGAGCCTGGATCGGTTCTGCCTGGGTGGGCGGTGCCCGGCCA  
 CGGGCAGCAGGGGCAGCGGTGCGGGCCCGAGCCGTGTCTGAGCCCCCTTGCCGCTGTCCCCACAGCTGTAC  
 TGGTTACCGGTGGAGTTTGGGCTCTGCAACAGCAACGGCGAGGTGAAGGCCCTACGGGGCTGGGCTGTCTCT  
 CCTACGGGGAGCTCCTGGTGAGGCCTCCCCACGCGCTGGGGCCTGGGTCCCCGGGGAGGTGACCCCTGCGG  
 TGCCTTGTGGATTCCAGCTCTCGGGAGGCTGGAGCGAGGGGCTGCCCTCCTGGGGGCACCAAGAAAGCTGGTC  
 TGGCCCCCTCTCCACACACCTGTGCCTGGGCCCTG  
 GGGGGACCCCTGCTGGGGGATGTGGGTGCACAGCCAGGGCCACCAGGGAGTCAGGACACGGGGCTCCCTTCCC  
 TCGGGTCCCTGAGACCCCTGGCCTCCCGCCAGCACTCCCTGTCCGAGGAGCCCGAGATCCGGGCCCTTCGACCC  
 CGACGCGCGCGCGTGCAGCCCTACCAGGACCAGACCTACCAGCCCGTCTACTTCTGTCTGAGAGTTTCAGT  
 GACGCCAAGGACAAGCTCAGGTGGGCGGGGCCCGGGGCCCAAACTGGAGGATCCAGCTGCAGCCCCGCC  
 TATGAGCCCATTTCCAGCAGAGGGAGCTGCTGCGGACCCACCGTCACAACCCCCCTCCACAGCTGGAACC  
 CCAGAAAGCCTGCGGAGGGGGGACCTGCAGGGCTG  
 TGGCCAGGTCAAGGCCAGGTGAGGCCAGGCTTTAGGGGTGAAGTCTGACTTTGTAAAGAGGGGGTGCAGGGT  
 CCTTCCAGCCTCCTCCCTCCGAGCAGCTGGGGCGGGGCGGGGTGCGATGAAGGCAGAGATGACGCAGCC  
 ACCCGTTACCCCTCAGGAGGCGCCTCCTGTCCAGCCAGGCTCCTGTGTACAGGGGAACTGAGGCCCCAGG  
 TGTGTGTGGGGGGGTGATTCTCACACAAAGCTTAGGGACAGGGACATAACGGCCTCTCCAGGGCACACAG  
 TCTGGAGG

## Contig 4 (3024 bp)

TTAANTCCANGTTGGCCCCACAAGTTTTCCCCATTGAAAAGGGGCCAGTTAAGCCCCAACNCAATTAATTGG  
 AAGTTAGCTCCCTCATTAGGCTCCCGAGNCTTTACNCTTTATGTTCCGGTTTCGATTTTTTGTGGGAATTGTA  
 GCGGATACAAATTTCTCTAAGNAACCAGCTATGCCCATGATTACGCGGTACAGTAGTTTATCAGTCCCCCCC  
 CCCATGGGACAGCGAAGGGAACCACTATGCTGGGGCCGGGTCTAAAGGGGTACCACCAGGGAGGGGCAGG  
 GGCTCCAGGAGGCAGGGCCACTGAGCGGTACCTGGTGGGGGGAGGTGGTGGGGCCACACCAGGAGTCTGTG  
 CCCCCCTTCCCGCCGTGGACATGAGAAGCAGGGGCCAGCCTGCGGGTCCCTGAGTTCAGCGCCCCCCCC  
 CCCCCCGCCGAGCAGCCCGGGTCTCAGCAGGCTGCTGTGCTGGGGCGGGGCGGCTTATGGRGCCGGGAG  
 CAGCCCCCCCCACGGCTTCAGAGCATCTCTGGGGCTCAGGGATGGACCGGGGTCTGCRGGCAGGTGTCTCT  
 TCGCGCCCCCTTCCCTGGGCTATAACGTGGAAGATGCGGCCCAAGCCCGGKCGGTTTGGCCTTTGTCCCCAG  
 CCAGTGGGGACAGCCTGGCCCTCAGGCCGCTCGTTAAGACTCTAATGACCTCAAGGCCCCAGAGGCGCTGAT  
 GACCCACGGAGATGATCCCGAGGCTGGCAGCAGGGAAATGATCCAGAAAGTGCCACCTCAGCCCCAGCCA

FIGURE 6, CONTD.

TCTGCCACCCACCTGGAGGCCCTCAGGGGCCGGGCGCCGGGGGAGGCGCTATAAAGCCGGCCGGGGCCAGC  
CGCCCCAGCCCTCTGGGACCAGCTGTGTTCCAGGCCACCGGCAAGCAGGTCTGTCCCCCTGGGCTCCCGTC  
AGCTGGGTCTGGGCTGTCTGCTGGGGCCAGGGCATCTCGGCAGGAGGACGTGGGCTCCTCTCTCGGAGCCCT  
TGGGGGGTGAGGCTGGTGGGGGCTGCAGGTGCCCTGGCTGGCCTCAACGCCGCCCGTCCCCCAGGTCTCTAC  
CCCCGCCATGGCCCTGTGGACGCGCCTCCTGCCCTGTGGCCCTGTGGCSCTCTGGGCGCCCGCCCGG  
CCAGGCCTTCGTGAACCAGCACCTGTGCGGCTCCACCTGGTGGAGGCGCTGTACCTGGTGTGCGGGGAGCGC  
GGCTTCTTCTACACGCCCAAGGCCCTCGGGAGGCGGAGAACCTCAGGGTGAGCCGAGGGGGYGTCCCCGGA  
GCGGTGGGGGAGTTTTTAAGGAGGAAATTGGTAAAGTGACCAACTCCCTGGGAGCTGAGCCCAGAGACACC  
CCTCCACGCCCCYGGTCCCGCTCGAGAAGCCCCCTTCCCTCCCTCCTCCCG  
AGGCGGCTCCAGGGAGGAATCTTACGGAGTCAAGGCCCGGGTGCCGCTGGTCTCCGAGTGACATGGCCGTGGT  
GTCCRTCTGCCGGCCACATGCCCGTGAGAGAWGCCCATCCCCCTGGGAGGGGGCCCGTGCCGGGAGGC  
GGCGGAGGCCCAGGACCGGTGGCTGTGCGGCTTCCACTCCAGGGTGGGCGGGGTGGGGGTGGTGTCTCT  
GTGTGACCGGCTCTCCCCGAGCAGGTGCGGTGGAGCTGGGCGGAGGCTGGGCGGCTGCAGGCCCTGGCGC  
TGGAGGGGCCCCGAGAGCGTGGCATCGTGGAGCAGTGTGCTGCACCAGCATCTGTTCCCTCTACCAGCTGGA  
GAACTACTGCAACTAGGCCGCCCTGAGGGCGCTGTGCTCCCCGACCCCAAAACCAATAAAGTCTTGAA  
TGAGCCCGGCGAGTCTGTGGTCTGTGTGGCCTGGGGCGGGGCCCTGGTGGGGAGGGGCCAGAGGCTGT  
GGGGGGCTGCTGCGACCCCTCTCTGCTCTGCGCACATCGGCTGCTCTAAGCTTCTCCACATGCATCGGGT  
GCCCCAGGCACATGGGCACCGGGGGACAGGGGCCAGGGCAGGGCCCTTCAATGTGGCGAGCTCTGGTTTTT  
AGGGCTCCAGACACCCCTCCTGGGTGCCACTGTGCACAGGGTCACTCTGAGGGTACAGGGCACCCACCC  
AGACTGCTCTTGGGCACAAATAGCCAGGGGCTTCTTGGGCTGGCTGCRGTCTGGGAGGTGAGAGGTGA  
CCCCCGGGGACCAAGACCTTGCCAGCCTGCCAGTGCAGGCAACCAATCTGCACCTTTGCTGAAGGTTT  
CACCCGGGCCAGCACTGGGGGCGGCCGGGCTAGAGCTGGGCGCCCGGGCCAGGGACTGCACACCCGCCAG  
AGGTGGGCTGAGGGGTGGCAGCAGGCTCTCCGCTGGGACCCAGCCAGCTGGGCGAGTCACTCTCAACACG  
AGGCTCTACCTGTGTCTCCCTCCCCACGGCCACACAGACACCCCTGGGGAGAGTCAAGGCCCCAGCA  
GGCCCCGCCCTGGAGAGGAGGCCAGGGCTGGGCGGGGCTGGCCGGCCGGACACTGGACCCGGAAGGGGG  
TAGGCGGCTGGGATGAGTGGCGAGCTGTCCATGGGAGCACCAGCGGCCCCATTGGCACCACTACAGGCAGGG  
GCACCTGCAGCAGCTGAGGTACGTGGGGTCCCCGGAAGTGGTGGTGTCCGGCTGCCCTCTGGGAGGCAGCGG  
CTGAGCTTGTGGTCTGCCAACCAGGGAGACCCGTGACCACCTGCTGCTTCCCTCCCCCAGGGCCAGCA  
GACTCTTGTGGGACTCGGGGCCCCCTGAGCCGCCCCACTCGCAGGACTCACGGGTGTGCGGTCTGGGTGAG  
TGGGGGCTTGGGAGAGGGTCACTCTTGTCCGTGGGTGGGGAAGGCTGAGAGTCATGGTGTGACAGCGCCCTC  
GGCTGCCGGGTGGGGGTCTCCCTTCTCCGAGCCAGATCCCCGGGTAC

Contig 5 (1730 bp)

CGTACCCCGCAGAAGCCAGGCCACAGGCCTTGGCTCAGCCCTCCACCCAGGCCACGTTCCGCCCTTCTG  
GGAAGTGGAGGACAGCCCGCCCTCGCCCTCGGACCTGGCTTCTGTTGCCCTGGCATCTGGCAGTGGCCGGCAG  
CTGCGTTACGCCCTGGATGACACCTGGCGTGAGCGGTGGGTCCCCGTGCTGAGGGCAGCCCCACACAGCT  
CTGCTCACTTGCCTTGTCTGCTCCGCATCCCGTCATCACATGCCATGCTGGGGCACCGTAGCGCCTTGC  
CCTGTGTGGCAGCTGTGGCAGTGTCTTCTGATGGGAAGACTGAGGCTGGGGTCAAGGCCGCTGCTGCCACCC  
TCTAAGGACATTCTGCCGTGACAGTGCCTCCAGG  
CTGGCCCCCGGATTGCATCTGCTTCTGGCACGATGAAGTGGCACCTCTGCCTGACCATTAGGGCTGTATTT  
GCCTTCTCTGTGGCAGTAAATATTTACTGTCCCTCCCTGTTCTCCAGGCCCGANCCAGTTCTTGGGGG  
ATGGGAGGTGGACACAAAGGTGCCAAGCAGCCCCCTGCTCTTGGGGCCAGTGTCTGGTGGGGCCAGCCT  
GGGAAGGAGGAGCGAGACTAGGAACAGAGGCTGTGTTCTTCTGGAAAAGGCCCCCTGGCAGAGTTCCGGCTGG  
TGTGTGTCCAGCTAGGCTGTGAGTCTTCAAAGTGGGGAGCCCCGGCCCTGGACCCAGGCAGGGCTGCACCCCT  
GGTGCCAGTGTCTACTGGGTGGGCACCTGTCCCC  
ACCAGGCAAGGTGGTCCGAGCGGTCAATCACAGACAGAACCAGCAGAGGGCGCCAAAGCCCCACTTTTGACAA  
ACTCCCCCTCGCCCTGAGCCGAAAGTCCAGGCGGCAGGTGGACCTCTCTGCAGGGCTTGCCACCCCTGCTGC  
CGCTTGCCAGCACTACAGGGGCTGCGGGGGGTGCCAAGCCGGCTACCCTGAGCTCTGGAGGCGATGGA  
GTTTAGGAGGGAACGAGGGGACTCCTGGGGGTGACTTCTTTCAGCGCCACATTGCGGCCAGCAAACCGAGG  
CTGGAGGAGGCCGGGCACCTGTGCCAGCTGGAGCCTTGTGAGGGTCTCAAGGCTGGGGAAATTGAGGC  
TGGGGGCTGGGGGTGTCACTGTGCGGCCAGGAGG  
CCCCTCGCTCTGATTGGAGCCGCTCGGCCACTTGAGCCAGGAGGCTCACATGAGGCGGGGGCTGCAGGGACA  
GGACCTCGGGGCCCCGGGAGGCTTGGAGGGGTCCAGCTGGGCCAGGGTTCGTTCTTCCGGGTCCATGTC  
CACCGCCCTCCCGCTGCTGGGAGGAGGAGGTCCAGGGCAGAAAGAAATCCGTGGGGATGGGGGGTGGTCAG  
GGGTCTGGGAGCTGTGGAACAACAACAGACAGCGAGGTCTGGGGCGCCCGGCCCGCCCTCCGGCA  
CTGTTGTTTCTGGCCGGGGTGCAGGGACAGCGAGGCAGATTCTTCAAAGTGGAGACTGCGGGGGGGCCCT  
CGGGTCTCAGCTACCCCTGAGCTAGCCCGCC  
ACTCGGCTCCAACTCCCGCAGGCCCTGGCACGGTCTCCAGGAGTCCACTGAGGGGTCCCCAAAGCTGCCAC  
CAGGAGCTGGGCTGGGTCTGTACACACCCACCCACCTCCAAGTCTGAGATATG

Contig 6 (4833 bp)

ATGTGAGCTGCACAGCATGAGCCCTCGGCCCACTGCTGTGGCCTTGGCGACATTGAGGTGTGTGCCGCCAG  
GGCGACCACACCTGGCCTCTCAGGCTGCCGTACAGAGCGGCTGGGTCTGAGGAGGTGCGGGGCTCTGGGG  
ACCGCTGGTGTGAGTTCAGGACGGGGTCAAGCCCTCTCTGAAGGTTTGGTGGGTGGCCCTTCTCTTAT  
CGTGATGACAATACTGATTTCTGGAAGAGCCAGGTGTTTCTGAGGCTGTGGTGTCACTTCTCCAGTGGCCA  
CAAGGTGCCGGGCTCGGGTCAATTGAGAAGCCCTGCGGGAGCGGGTGTATGCGCCAGATTCACTTGCCT

FIGURE 6, CONTD.

CCTGCGGGTCTGGGGTCAGGACGTGGTCCCCAGCAGTCTGCTCCAGAGCCTGTCAGTGATGTGTGGGATTTTA  
CCGCTAGAACACAGTTTCTCTGATTCTCAGAAACCAGCAGATGCTTTAGGAGGGGCGTGCAGGTTTACCTG  
TGCTGCANNGCCCCCTGCCACCTGGTCGGAGCCNCAAGACGGCATCTAAAGATCAGTTCCCTCATCAGTTC  
CGCAGTGCTGGGGTGGGGGCAGATGAGAACCTCAGGGCTGGGCGCAGAGGTGGGGAGCCCGCTGGACCCCGA  
CACTGCAGGGGGGCTCCCCCTTGTAGGAAGAACAATGTGCTTTGCCACCCAGCCCTCTCCCCAGGGTGCCC  
CGAACTGTTGCTCCTAAGACCTCTGGGCTGTGTGCTGTAATTTCTATAAGTGGCCACCAGGTGTGAGAGGAGG  
CCACTTAAGCATCCATGTGGCGGAAACCTGGAGCTGGGGGTTCCTAAGGGTCCCTCGAGTGTCTCCTGAATAA  
ATAGGCGCTGACCTGATCCCCAGGAAGGGATAACCTCTCCCAGGCCCTAAGAGGCAGTGGGGCAATGAGGTTT  
ATGTGTCCACTGTACCCCCAAATTTGTCTCTTCCCTTCCCTCTACCCCTGTGTCCCCACCCTGGACGATACACGGA  
TGCGCAGGCTGCGGGTCACAGCCCTCACAGCCCCAAAGCTGCAGGTCCCTGCCTCAGGGGCACCGCAGCTTGGC  
TGGTCCCCCTTGGGTCTCCCCACCCTGACCCGTCTCTGCTCCCCCTCCCTTTGCTTAAATGCTCTGCGTTTC  
AAGGTTCTGATGGAATAAAATAGCCCTGCACTGGTGTGTTCTCTTTGGGGCTGTGCCAGAAGTGGGAATTC  
GACCAGGGCAGAGCTCAGATTCCACATACTGTGTAGGGATGGCAGGTGCCACATTTCCAGGAGTTTCATTGG  
TGGTTTGTAAATGCTACTTCCGTTTCAGCCCCCTCAGCTGCCACCTCCCTCAATTTAGGGACCCCCCTTTGG  
CGGGTTGCCCATGGAACCACATCATCTGGCGTGGGGTGAGCCCTTTATCCTCCCTGGCCCCACTGGGAGGGTT  
TGGGAAGTCCCAGCTAAATTTCTCCGTAGGGACCTGGAAGGAGCCCTTGTGACATCTGGGCACAGATAAGAG  
GTAGGGGGCAGAGCCGTGAACACTTGAAGCTGCAGAGCCAGAGCAGAGCCAGGAGCAAGTGAAGTCTC  
CCCACCCCAAGAACTGTGGGCTGCGTCACACACTCCCCTGTGTGCCCTGGACCTGACAGGGCCTTTAGCCT  
CCCTGCATCCCTCCCCACCCAAAGACCCAGTGAGGCACCCCACTTGGCCCTCCTTAGTGTGTTATGGCTCTG  
GGGCATCTGCATTTTGTTTAGGACACCCCCAGCTAGATTTAAGTCCCCCAAGTGTGACTCTTTCTCCACTG  
AAAACCCCTGTCCCTCCCAAGGGCCCTATCCCTTTAGCTGAGCCAAGGAAATTCAGGAGGGGCTTGAATG  
ACAAAGGAAGAGGGGAGAGTTAAACCCCAACACTGGCTGGCAAGCTGGGTGGGGTGACACCCAGGGTGCA  
GGGGTGCAGTGAAGGTAGCGGCTGGTGGCTTCTGGAACTACATGTGACTTTGCCATTAGGTGAGTCTTTGC  
TTTGCCCCCTGCTCTATCTGCAGGCTTATGGAAGAAGTTTAAATTTCCAGGGACACTGGTCTAACAGGCAGC  
GCTTGTATCTGGGCCCTTCCCCAGCTGCTGACCACTCTGAGTCTGCGCCTTAGTTGGAGTTTGGCCAAGCTC  
AAGAGGCTGTGGACCCAGTCATCCCACCCAGGGGTGCCTGTGGGCAGGACGCTGCTGCCCTGCCATTTGCTGC  
AGTATTGTCACTGTCCGGCACACACACATGGTGAGGGGGTGATCAGGTGCCACTGGGGAAGGGAGAAAA  
CTCCCAGGTGAGTCCCCCTGCCTCTGGAAGCAAGATGGACATGACCGCACTGTGTTGACGCTGCATTGGGAGGC  
CCCGAAGAAAGATTTTTCTGATCTTTCTCGAACCTGCTTTTCCCCATCATGCCCGCCCCCATTTTACCCGT  
GCCACGCCCACTGGTGTGCCGGGGTGTCAAGTGAAGTGAACAAGTGTCAATCTACTGAGGCCCTGCCACTCTCC  
ACCCCCACATAGTCCCACCTCCCAGCTGGCAGGGAGAAGTTCAGCTAATGCCCATGCCACAAATGCTTT  
TCTGTACGCTAGAGCTGGACCAATCTCCACCTGTAACATGCTGTGCCCTGGCGTGGGAAGGTGCCAGAGC  
CAGTTGCCCCAGCAGCCCCAGAACCCTAAGTTGGCACAAAGCTACCCAAATTTGGAGGGGCTTGGGGAAGGG  
CATGGAGGGGATGAGGAGGTGAGGGGCAAACTAATTTAGTTAGCATTTGAGCAGGTGCCACGCTCAGCGTG  
GAGAGGCTCTCTTGCTTCTAGGGACCCATTATGATGCACACGCTAAAAGCGCCCTTACCATCTCTCCAGCCT  
CAGCTTTGTCCCCCTCTCCTCCTCAGCGGCAACCCGGCTGGAGGGTCTGGCCACTACAGCCAGAGCGCCCC  
TACTTTGGTGGCAGTCTACTATTGGCCCAACACGCGGATCACCGGCCAGGCAGTTTTCGGCAGAGAGTCTGG  
GGCACCAGTGACTCCCCCTCCTCTTTATCCACACCCAGGAGCTTTCAGGGACTACACAGCGACTAGAGGGCA  
GGTAACTGGTCTGCCCTCCCTAGGGCTGCCCTCAGAGTGTGTGAGAAAAGCTGCGATTGAGTGTGGGTGC  
AGGTGGGCTGGGGGCTTGGGGCAGCCAAACAGGAACGGCGGGACCTCTGCTTCCAGAGGACCCAGATCTGGC  
AAGCTTCGACTTTGGAGGGGACAGGAAAGACAGGTGGAGAGGGGACACTTCCCTCTTGTACAGACGCCCAC  
CCGGAGCCACAGAGGCTTTTGAAGGAAATAGGTTTCCCTCACTAATGCAGCAGGCAAAATGGGAGGGGCA  
GGGGTGGAGGGTAGTGCCCCCGCCCCAGCAGGAGGGGACAGCTGTTTCTGCAATGTAAAAAAGCAGGGTTT  
TTCTGTGTGAGAAGTTCCCTCTTGCTGCATGTCCCCACCCCGCCACCAAGACAAACAGGACACTGTGCAGA  
GGGGCCAGAGCCCCGAGATTTTGGAGTGTGTTTTATATGCATATATACCATTTTGAAAGCAAAGCTTCCCTCT  
CCCTACTCCCTACATGTCCCCCTTACCAAAAAATCCACACGTAAGTGGAAAGGGGAGTGAGAAGGACGA  
CGAAGGGGCACTGTCCCTCCCGTCCACAGCGGGACTTAAACGTACAGCTTTTGGCCTCCGGACAGTGTGC  
CGCCCCCTGGCCCCCTCAGCTCCCCCTGCCCGGGGGCTGAGTGTGGGGCCAGGGCCTGTCTCCAGGCATGC  
ATTATTTTGTGCATGAAGGTTTTGTCCCGCCCAACCCAGGCTGGTGTGGGGGAAGGGGTTTATTGCTCCAAA  
GAAGCCCATCTCCCCCTCAGCCACCTTTCAGCCGCTTTCGCAAGGCAGAGCTGTGTCTCTGCTGTGTGCTG  
GCCCCCTCCTTGCTTCTATTCAAGGTGGAAGTGTGGGGGAGGAGAAGAGTTTTATATTGTGTCTGTGATC  
CCCCGAGGCAGGGCATTTGTGTGCGGCCCCCAGCCCCAGGCCAGGCAGATGGGGCAGCCTGCCCGACAGA  
AGGGTCTCCTGCTGCTTGGCTGCAGGAAACCCAGCTCTGGGTGAACCGTGGGCACCTTCTTCTCCATGCC  
CTGTATTTAAAGAGGAGAGCTGGGGGGCAGAGGCACAGGAGGGGAGCCACGGCCCCAGGTCTGACAAGT  
GACCTGCGGGCTCTCCACCCAAGAGTCGGGGTGGGGGGCGGATTTGGTTGAAAAGAGAACAATGAGAAC  
ACACTCTTTATTTTCCCCAGGGGCGGAAGAGTCACCCCTGAACCTTGGAGCAGCAGCAGCGGATTCCAGCCCC  
AGCCCCAGGGCCCCACATCTCTCGGGCTCAGCCGCGCGCCCCAGCTGCCCCCAGCCTGAGCTGCAGCAGGC  
CAGGGCTGCCCGAGACCCACAGCCCCAGGTGAGCTGTGTCAGCCTGTGGCCAGGAGATCTCCGCGGCTCAG  
AAGTCAAGGCGGGCAGCCACCCAGCCACAGCGGTGAGTGTCTCCAGACCCAGGGCAGGGGCCGGTGTCCCC  
CGGCACAGAGAGCTGTGCTGCAGGCCAGACCTCCAGGCCGTTTTAGTTCCCATCTCCCCCTGGGGGAGGGG  
TGGGGCTCAGAGGGGCTGGGGTGATCCGCAGAGCTGGGGTGACAGGCTCCAGGTGCCTCTCTCCAGGCCGG  
TGGCCCGGAGGGGGG

Contig 7 (2014 bp)



FIGURE 6, CONTD.

CTGGTTTCGCACTCCTCCGGGGACTGTTGAAGTACCCGAGAGCGCNCGCGGAGCGCCGGGGCGAGCGGGGGTG  
 GCCGCCGGGGGTGCTCCCGGGCCCCCGGACCGAGCCAGGGACGAGCCTGCCCGCGCGCGCAGCCGGGGCCGCGG  
 CTTCCGCTAGGCTCACAGCGCGGGAGCGCGTGGGGCGCGGCCGCTGCCGGGAGTCCGCCTGCCTCCTCGGAGG  
 CGGCCGACCGGGGAGCCTGGGGGACCCCGAGCGCCCGGGGAGCAGCGCCCCGACACGCCCCGGGCCGCTCTCG  
 GCTTCCCTCCCTTCCAGCCGGCGCCCGCGCGCGCGGGCTTCGGCACCGGGGCGCTCTCAGTGGCAGGAGAAGCG  
 TGGCTCCTCCCGCGGGGTGGGGGACCCGAGGAAACC  
 CGCACCGCCTGGAGCCGCCCGCGCGCGGCCAGCGCTCGCGTCCCCCGGGGAGGGCGCCACTGCTCCGCGCGCG  
 CGTCCCCCGACGCCCGCGCGCTTCCCCGGCGGGCCCCGGGATCCTAACCTCTCTCTCGTCCGAGCCCCGCA  
 CCCCAGGGCTCCAGGCCCCCGGCGACTTGCCCGCTCCTCCCAATTGCAGACACGACTTTTCTGGGACCTCCC  
 AAAGGACAGCCTGGCTCCAGGGTCCCCCAGATACATTACCATTTCTCCAGATCACAAAGTGGGTTTTTTCGGGC  
 ACTAACTTCCAGAGACCTCAAAGCACATGAGCCCCCTACTGGCTTTCCAGGTTTCCACTAGTGGCTCGGTCC  
 CCACCTCACTGGGGATTGTCTCCAGGCTCTTCGC  
 GGTGTGATCCACCCATTTCGCGCCAGGTCCCGCAGTGCCAATCCCTCCTCTAGAAAACCTTAAACACTGACTC  
 CTGGTCTCGGGGTGAGGCTGCCAATGTGCTGACTCCCCAGAAAGTATACAGTGTCTTCTGGCATTTGGG  
 CACCGTTCCCCCAAACACGTGAAGCTCTTTCCCGCGTCCCCATAATTTGGACGCCAGGGGACCCAAAGCT  
 TAGCGCCCTGTTTGGCTCCCCACACCGGAAGCCCTGCTCCCTGGGGTTCACGACAGTTTGGGACTTTATC  
 TGCCAAAGTTCACAAACTGATTGGCCCCAAGCTGGGGTCCCTAAATTGTACACAAAGAACCCAGCCCCCCCC  
 CCAACTCCAGTACAGGAAGCGATGGCCCCAGGGA  
 CCCTCGGAGTTGGAACGTGGCTTCCTAAGCCTTCACCAAAATTGAGGCTTTCCGCGCATGGCGCGCTGATGCC  
 CTTGCTGAATCAGAAGCACTCTGCCCTCTGATTCTGCTTTCCACAACCTGAGAGCATGATTTCTGGTCCCC  
 CAAACTCACTGAGCAAAATCTTTTGTGGGGGTGCAAAAGATAGGAGGCATTTCTCTCCGAGCTCTCCAAA  
 CTCCCTTGCTAGTAATCAAGTTCCCTAAACTTAGACAGAGCTTCCAGGCCCCAGAGGCACACAGAGCCATT  
 ATTGGAGCTGCGTTTAATGATGACAGGGACCATGGGTCTGCGCTCCCCAAGTCACAAATGCCCGAGGTAT  
 CCTTGGCTCCAGCCAAGCCCAAAGCAAACTCTTGC  
 ACAGATCCCATATCTTGTATGTCAAGCGCTTTGCGTGTCCAGTAAACAAATAGTCTGAGTGTCTTCTCCAC  
 CTCATAACATTCGGAATATTAATAAATTCCTTGGGCCCCCGAGCTGACAGACAAGAATCCGGGCTTCTCTAA  
 ATTCAGAACTGATTCCCAATCCCGAGGCCAACGCCAGACCTCTCCCAATCTGGAGCCCTCCGACTGGACAC  
 ACTGGACTCCTAAGTATTACGCGCTGTCTCCAGGCACCCCAATGCATTCAAAGTGACGCTTTGGTACAGA  
 AAGGCACTGATTTCTTGGGTCCAAAGCAGCCCATGCACCCCGAGTACCCCAACTTAGTCAGCATTTCCC  
 GGGTCTCCCTCCGCACTGCAAACTCCCAACTGCGG  
 ACACCGGTCTTCAGGACCCACCGCTAGACGGTCTTAATCCCTTTTCCCCAGACCTAGATTCTC  
 Contig 8 (371 bp)  
 AGATTCAAAAATATTTTCTGGGGCTCCAAATTGAGGTGCTGCCTGCCAGTCTCCAAAATAAACTGAGGG  
 GTTTTTGTGTTGTTGTTTTTTTGTGTTGTTTTTTTTTACCTTCCACGAAACAATCCAACTTTTTTGGGA  
 CCATTGATTTATGGGTCCCTGACTTTATGACCCTTGCCCCAAGTCCCCCTAAATGTAGGCCATTTTCCACGG  
 GCCTCCCAAAATGAAATGCCCAGATCCCGCGAAAAAATATCCCCGGGTCTGGAATCCCAGGTATTACA  
 GGCTCGGGCTGACACCCCTCCTTGCTACTAACCAGGTTCCTGAAGTTTAGAGATCACTACCTAATGAACAA  
 ATCCAC  
 Contig 9 (2415 bp)  
 CCAAACTGGGGCCCTATCTTACTAGGGTCCCTAAATGCAGACAGCGCCCGGAAAAATAGGGGCGTTTTTTT  
 TCCTGTTTGCCAAAATAAACTAATTGAAACCAATTTTTAGAAATAAAATCTAAATGACCTTGATTTCTGCTC  
 GTTCTCCAAATGTACTTTTACAGCCAGGTTGCCCCAGTTTAGACGGTGTGCTTGAATCTCTAAAGCACC  
 CTGAGGATTTTCCCGAGGAAGCCACCACTACGGAATTTACTGTCTTCCGGGCCACAAGCCTCCAGGCC  
 ACCAACTTGGATTTCTAAACCTGGAATCAGCCTCCACTTCCCTCCGCGACCCCGAGGGTCTGCTCAGACCC  
 CCAAACTGCGCGCTGTCTTCTCCCCCAAAAT  
 TTATTTAGAGAATATGCCTCTCTCGGGTCTGCCAAGTTTCCGCTGAGACTTCTCGGTCTATCCCCAAATCC  
 TCTTCCCGACAGTCCGGGAGCCCCACAAGCTTACCGACCCACATGCTGGGGTCCCCCAACTTAAACGCGATC  
 CCCTGTCCTCCAGATTACCGAGTGATTTCCCTGGTCTCAGACTGGGACTCTTTTACTGGAGTCTCGAATTT  
 AGCCATTAAATCACAGTTCTCCACTCCGACGAGGCTCCCTTGGGTCCCCACGTCCGGGACATGGGTTCTCTTG  
 CCTGCAAAATCAGGCTGCTGACTTGCAATCAGGCCTTTGGGCATTGTTCCCCGCGCGCGGTCTCGGTTC  
 TCCCCCATCCCGCGCACGAGCGGCACTGGGTCTG  
 GGCCTCTTGGTGTCTCTACAAGTCCCGGAGCTCCTCGGACTTGGGAAGTGTCTTTCGTTCCCCAAATAC  
 ACTCGGCCCGGCGAGTGTGTCGCCAGGACGTAGGACGAGCTTCTCCCGCTCCAGGAAAACGACTGGGCATTG  
 CCCCCAGTTTCCCCCAAATTTGGGCATTGTCCCTGGGTCTTCCAACGAGTGGGCGTTGCCCGGACACTGC  
 GGACTGGCCCCGGGTCTCGCTCACTTCAGCGCGTCCACCGCCGCTGCAGAGCGCTCGCTCTCCGTCTCTC  
 GGCTCCAGCGCGCTTGGGACGCGAGCTCCGGGCTCCAGCCTTGCAGGTGAGTCCCCGTGCGCTCGCGTGT  
 CCGGCGCGGCTCCCAAAACCACTCGCCGCGCTC  
 CGCTGGGGCTGGCACTGGCCTCCGGCGACTGCCGGGACACGGGAGCGGAGCGGGAGCCTGCTGCAGGCCA  
 GCCGCTCGGCGCGCGCGCCCTGAACCGCGCGCGGCTTTGTTGCTCTTTGCAAAAGTGCACAAACCGTGG  
 GGAAAACGCTCGGCGGCCCCAACCGGCGAGGCGGCTTGGGAAGGAGGACACGCGGGAGAGGAGCAGC  
 CCGCTGGGGCGGCGAGCGCGCGCTCCAGCGCGCGGGGAGGATCCCGGAGGCGCGCGGAGCGCGG  
 GCGAAGTGATTGATGGCGGAGCGAGGGGCCAGCGGATCGCGGGCTTCCGCGGCGGCGGCCCCCTTCCCTCG  
 GAGGACTCGGGCGGCGCGGTTTCTGGGGCGGG



FIGURE 6, CONTD.

CCACACACACACATGCATTACACACACACACACTCGTGCATACACACGTGCGCGCGCACACACACACACA  
CACACTCTCTCTCTCTGTGGGATCCCTGAG

Contig 19 (500 bp)

TGGCTCTGGCATAGGCTGGCAGCTGCAGCTCTGACTGGACCCCTTGCTTG  
GGAACCTCCATATGCCGTGGAAGCGGCCCTAGAAAAGGCGAAAAA  
AAAAAAAAAACCAACAAACAAACAAAGCCAAACACACAGAACTC  
ACAGACACAAGAAGAGACTGGTGGTTGCCAAAGGTGGGGTCGAGGGTGGG  
AAAAATGAGGAGAGGGGGCAAAACACACAAACGTGCAGCCATAAAATGGT  
AAAGTCCCGGGGACCTCCGGTAGCGCGTGTGGGGACTCGGGTTGAGAACA  
CACCGTGATGTGTATTCCGCGAGTTGCTAAGAGTCCCTGTTGGAGAAACAA  
ATGCGTATCGACGTGTGGAATGAAAGTTAACCCGACCTGCTGCTGAT  
CACTTTGCAACACATACAGACATAGAATCATTATGTTTTACCCCTGGAGC  
TGACAGCGTTATACGTCCCCCAGCCTCAATTTAAAAACAGCGTTGCCGTG

Contig 20 (400 bp)

TTCATACTGTGCAATGCCAGCCTTAAATGCACAGAGGAGAGCATTAACTT  
CTTTGCAGAATCACTGAAATGATACTCATGTTTTGCAACTTGCACTT  
GGGGCTTATTTTATTGGTGCCGGAACAGCGCGGATGTGGCACCAAACTAG  
CGCCGCTGTTTTTATTTCCCTCGGTATCCGCGCTCTCGCTGTCTTCCCC  
CCCTTCCGCTTGACGTGAGGAAAGGGCTGAGAGGAGGAAAGTCTGCATT  
CACCATCTCCCCCTGCCCTCTGTTGTCATCCTTCACAGAAGTGGTGGCCT  
GTGCGGGGAAGTCACTAAACCTAGGCAGGTGTCCCGTGGGGTCATGCTTG  
TTACACCTTTGTGCACCTGGCCCAAGTTCTGGGTGGAGCGAGAACGTGGC

Contig 21 (559 bp)

AGCTAGCCCCCAGCCAGGGCCAGGCCTCTCCTGCCACCCGCCAGCCA  
GCATGTCTCAAGAGGAGGGGGCCTCTAAGGGATGAGGACCTGCTCCAGTC  
GGAGACACGAAGCCCCCGCGGCTCCTCCCGAAAGTCCAGCTGCGGCTTT  
CGAGCACGGCTGCGCCCTTCGTCAATCATTTACGCCACAGAAGTGAAAGG  
CGCTTTCTGTCGGCGAGGCAGGCGGGACACAGAATGGAATCCACCCCA  
GCGAAGAGCCGCCGTGGGTGAAGCGCTCTCTGGTGGGGACCGGGCCGGG  
AACTTCACATGGGGTGCCTGTCCCCATCTCCCCATCGTCATTACTGCAG  
GGCTCGGGCCACACCCGGAGCTGCGGGGGCCAGTGTGACACTGGACCT  
GGCTCCGTCCTATGATGTATGGGGGCGGGGCCAGCACAGGGCAGTGGC  
CACACCTCGGGCCTCCAGCACCAGCCAGGATGGCAGAGGGCCCCACCC  
ACCACGGGGCATGTACATCCAGAGGACCAGCTGAGCAAGGCTTGATANG  
GGCTTCAAC

Contig 22 (450 bp)

CGTGCAGGGACCCGTGCGGGCCTTCCTGTGGCCACAGAGAACAACACAC  
CATTATCTTCAGCCCCACCGCGCGGCTGTTAATGGGTAAACTGGGGCAA  
GGGGGCCCCCTGCCGTAGGCGCGGGTGGGGAGCGCAAGGCATGGCCTGTGT  
GCCCCAGCCCAGTCCCTTCAGGGCGCTGCTGTCTGCACCGGGGGCCCCAG  
GAAGCAGAGCACCAGCTTCTCCCTATTCTAGAACCAGCCCCAGAACC  
CTGGACACAGGATATGGATTGGGTGTGGATTAACTTTTCGCAAATGT  
GCCACTCCACACCCACAGAGGGGCCAGCAAACCCAGTCACTGCGCAGC  
CCATGCCCAGGGGGCAGATGGGACACGAGAGCAGCCCTCATCCACAGCAG  
GCAGGGGAGTGAAGTGGTGAAAACGGGGCGGTTCCACGAAAGTTAAGCA

Contig 23 (535 bp)

TGCCAGAGACCTCAGAGCTGGGCTCTGCCTTCCCGGGCTGACACGGAGGG  
CTGTGGCTTCCACACCCAGGCCACAGCCAGCCTGCCAAGTCCCTGAA  
GTGTCCCCAGAGGTGGCCCTGCCTCCACGCCCAACATCAGGCCTGCTGCA  
GCCCTGGACGGCCCCCTGTCCCCCGAAGCCCTCGGGGCTCTCTCGCGTC  
GCCTCTGGGGAACCTCGGTAATGTGGCCCAGCCGTGCACTGGCCGGATC  
ATTTGCTCAGGGGGGCCCCAAGGCAGGGGGGTGACACATCCGCAAGTACCG  
CATATGCACAGGATATGGATTGGGTGTGGATTAACTTTTCGCAAATGT  
CTCTGCCGGTACAAATATTGTTTCTAATCCTCTGCCTCCCTGAGCCGGTG  
AGTCTGCCCGGAGCTGCGGGGAGCTGGCTTGCTGAACCTGCCCTGGCCC  
CCACCCCCAAGGGAGCCCCCGGCCAGTGTGAGGGCAGGAAGCTTGGGCA  
CAGGCTGCAGAGGCCAGCGCTGGCCTCAGTCACCT

Contig 24 (868 bp)

TATTGAAGACCTATCATGAGTTCACAGAGCGGAGGGGTGGAAGCAGGGG  
CCTACAGCCCACTCCCCATCACTCCAGACCCGTCCGGGGCTGGTGTCCCC  
TGCCCCCTACTCTGTCTCTGGTGGCGGACGCTCGAAGGAGGCACTCTG  
GCCTGGAGCCTGGAGGGTCCCTGAACTCCCGCTGCCACCTGGGCCCTCGG  
GCTCCTCCTGCGCTGGGACCCGCGGTGGTGGGAAGCAGCCCTGCTCAGTG  
GGAGGAGGCAGGGCTGTGGCCGCCCGCACGGCCCTGGGGGGGACGCACG

FIGURE 6, CONTD.

CAGGACGCANGTGGGCGTGTGTGAGTCCGTCTACACGTCCAGCCAAGGGC  
GGCCGCGACCGGCCAGGGTGGGCAGCCCCAGCCTCAGCAGGGCGCTCTCT  
GGGGCTCAGGCTGCGCCGACGGGAGATGAGGGGTGAGGCGCAGTCTGGGG  
CTGCTGCCGAGAACCTCGCCAGCTGGCAGCTGGGCACAGGGAGACCTG  
TACTCCAGAACCTGAGGCTGGACGTCCGAGACCCGCGTGCCGGCCTCTT  
GGGTGCTTGGTCAGGGTCTCTTTCTGGTTGTGGGCAGAACCTCCTCAG  
CGCGTCCCTGTCATGGGGTGCTAATCACGGAGTAAGGAGCCAGAGAATGAG  
GCACGGAGTATCCAGTGTAAACCCTGGAGTATGGAGACGGGAGTACTAAT  
TGTGGAGCATGGCTCTAAGGAATGGAGTATTCGTACGGAGAACGCGGGG  
CCGGGTGAAATACGGAGAGCGGCGTACGGACAACGGGGACGGGGTATCCG  
AAGGGGAGGATGGAGTATCGGCCGAGGGTGGAGAATGGACACTAGAGGA  
TGTATANNNGGCGTCAAT

Contig 25 (500 bp)

ACCAGTTTCGATGAGCAATCCCAGCGGCGTAACATTATGGTGCAGCCTG  
GTCAATGCCGGTGGAGTTTGAACCTCCACGCGTGGCGATTGTGGTAGATA  
AATCGACATGGACCAGGGAGTTGATTGAACATAACGGTAAATTTGGCATC  
GTTATCCCGGGCGTTGCAGCACTAAGTGGACGTGGCGGTGGGAAGTGT  
GTCGGGGCGTGATGAAGATAAAATTTAATGCTATGGCATTCCGGTTGTGA  
GAGGCCCGGTATTTGGTTTGCCTCTGGTCGAGGAAAAATGTCTGGCGTGG  
ATGGAGTGTGATTGTACCTGCGACTTCTGCGCAAGAAGAATACGACAC  
GCTGTTTGGCGAAGTAGTATCAGCAGCGGCAGACGCACGGGTATTTGTCTG  
AAGGCCGCTGGCAGTTTGATGATGATAAGCTCAATACGTTGCATCATTTA  
GGTGCTGGGACGTTTGTACCAGCGGCAAGCGTGTACGGCGGGTTAAGC

Contig 26 (900 bp)

ATGTTTGATGTCCGCGCGTGTGTAAAAATTTACGCTGCTCGGTTCTTT  
GGCTTCGTCCACCACCGGAAAACGGACAAAAATTTCCGTACATACCTTTTT  
CTTTAGGCGGAAGCCAATGTCTGAATCTTCAGTAAGACTCTGCACGTCTG  
AAAGCAATACCGTCACCGTCAGCTAACAGTGGCGTCACGGCGCGGCGGCT  
GAAACAGGTGCCGACGCTGCGCTGGGCACTTGTCCGGCGAGGGCTTCAC  
GCACCGGAACATCTTTGCCATGCAGCTCTGAAACTCATCAATGTAAGTC  
ATGCTGGTGAAGTGGTCCATTTCGCGTTCGAACGGATACACGGGATCTG  
AATCAGATCTTTACGCTCGACCAGATAGTTGAACAGACGCAATTCATCTG  
GTGAAATCACATCTTCGGCGTCATGCAGAATAAAACCAGCAAAAGCGAAA  
TTGGCGCTACGCTCAAATTTGGGTGATGGCGTCCAGCACGTTGTTTCAGACA  
GTCGGCTTTGCTGGTGGGGCCAGGACGCGCGCAGACTACCTTATGCACAT  
TCGGGAAGCGAGCGCACACTTCGTCAACATCACGCTGAGTATCGGGGTCTG  
TTGGGGTAGGTGCCAACAAAGATATGATAGTTTTCTAGTCGAGCGTGGT  
CGCCGCCAGCTCGGCCATATTGCCGATGACGCCCGTTTCATTCCACGCCG  
GAACCATATCGCTAACGGTTTTTTCATCTGGTTTATACAGTTTCGCGGTAA  
CTCTTAAGGACCTAGCCCCCTATGCTGAAATGCTGTACCTCGTGCTTG  
TACCCAGTATACGACATCTATAAAAAATCGTCCAGCCCGCTGATGAACA  
TGATGACCGCTAACGTTATCGCGATTACTTTTAAGCCGTATAGCCAGGTA

Contig 27 (500 bp)

AGCTGGATGCCCCAGCTGTGGTCCCTTCCCTTCCCTCAGGGCAGGTTCT  
GTCCCTCTTGCAGCCACCGTCACTGTGTGGACAGGTCTGCACACCCGCC  
GTCCACCAAGAGCGTGGCAGGTCCCTGGGCACGGGCGGCTCCTGACGCA  
CCATGTGTTCAAGGCAAGAGCACTGGACAGAGGGTCCAGACGTCCCCTTG  
TCCTGCTCAGGCTGGGCGGGGCGAGCCCTGGCGGGAGAGGCCCTGGGCA  
TCAGAGCCTCTGTGGCCTGGAGCTTGGCGCCCTGCCCTCCCCACCTCCGT  
CCTGCTCCTCGCCGCGTGCACGGACCTCTCCCGCCCCCAGGCTCATT  
ACTCTTAAGGACCTAGCCCCCTATGCTGAAATGCTGTACCTCGTGCTTG  
TTTTCATCTGTTTATTACCTTATCTTCATTCCCTGCTTGATGATATCTGGT  
TATTCTTTATTGATTATATATATCTTGTTCGTGTTTTATAGGACACTGT

Contig 28 (450 bp)

AGTGCGGTCCGGCGCTGACGCTCAACACCGTATTTCCACGCGACCGC  
GGATTCAACCTGGTCACACGGACGCCATGTAGACATGTTCCGGGGTTACGC  
GCAGAGAAGCGACCTGCTCAACCGGCTGGTGAAGTCGGGCGCTTTCGCCC  
AGACCGATGGAGTCGTGGGTGTAAACCATCACCTGACGCTGTTTCATCAG  
CGCAGCCATACGTACGGCGTTACGTGCGTATTCACGAACATCAGGAAGG  
TGGAGGTGTACGGCAGGAAGCCACCGTGCAGGGAGATACCGTTAGCAATC  
GCGGTACATACCGAACTCGCGAACACCGTAGTGGATGTAGTTACCCGCAGC  
ATCTTCGTTGATTGCTTTAGAACCAGACCAGGGTCAAGGTTAGACGGCG  
CCGGGTACGAGAACCGCCGAGGAATTCGGCAACAGCCGGACGAACGCT

Contig 29 (450 bp)

FIGURE 6, CONTD.

TCAGGCCAATCTGTCTGGTCTCCAATGGGGACAATTTGGTTCTTTAGGCT  
TCTGTCCAATGGTCCGAATGGCCCACTCCCCGGGCGCCGCAAGGGTCC  
TCTGTGCTCGGGTGGGCTGGCAGGACCGCCCCAGGGTCGTGCCAGCC  
CCGTCACCGGGGCCAGAAGCTTCGGGCTCTAGCTGGCTAGTCGGGCTG  
CTGTGCAGGGGGGCTGCGCTGGGGGCAGAGGCGGGGGTGAGGTAAACCTC  
CCAGCCGCGGGGTCCCTGCCGAGCCCTAGGCGCCGAGACGGTGGCTG  
GGTCGGTACCGCCAGACCGAGGGCTCGGGGCCGGGTGACCCAGCTG  
TCGCACACGCTCGCAGCTCTCTTGCTCATCAGGGCTCATCCCTCTGGACC  
TCTCTACTGCCCCACCTCACCCGCTGGACCCCATGAAGCCCCGCGGA

Contig 30 (600 bp)

TAAAACTAGCTCTAGTAGAAACATTTTATTTAAAAATAAAAAACCTGACT  
ACGTCGGGAGTTCCCGTTGTGGCTCAGTGGTTGACGAATCCGATGAGGAA  
CCATGAGGTTGCGAGTTCGATCCCTGGCTCGCTCCGTGGGTGAGGATC  
CGGCGTTGCCGTGCGCTGTGGTGTAGGTTGCAGATGAGGCTCGGATCCTG  
CGTGGCTGTGGCTCGGGTGTAGGCCGGCGGCTACAGCTCTGATGAGACCC  
CTAGCCTGGGAACCTCCACATGCCCTGGGAGTGGCCCTAGAAAAAGGGCA  
AAAGACAAAAAACAAAAAGAAAAAGGAAATATAAATAAAAAAGACTATGT  
AAATGAAATTAACGACTGCCTAGGGTGGGATTTACAGCATGGGAAGTACA  
GCATGGCCGTGACAGTGCAAGGGTGAGCGGGAAAAATGGAATAGGTTAG  
GTGAGTTTCTCCTGCTATTTGTGATGTGGTCTGCTATCGCTTGAAGACGG  
ACTGCAGTGAGATAAATATGTACAGTAAGCATCCGAAAAACCGCCAGAAC  
GGCAAAACGAATGACTCCAAGTAAGAACCCAAAAGAGAAAAGGAAATAAT

Contig 31 (450 bp)

GCGCGGGCGTTCCGGCTGGGGTATTTAACGTGGTCACCGGTTCCGGCGGGC  
GCGGTTCGGTAACGAACTGACCAGTAACCCGCTGGTGCGCAAACTGTCGTT  
TACCGGTTCCGACCGAAATTGGCCGCCAGTTAATGGAACAGTGCGCGAAAG  
ACATCAAGAAAGTGTGCTGGAGCTGGGCGGTAACGCGCCGTTTATCGTC  
TTTGACGATGCCGACCTCGACAAAGCCGTGGAAGGCGCGCTGGCCTCGAA  
ATTCCGCAACGCCGGGCAAACCTGCGTCTGCGCCAACCGCCTGTATGTGC  
AGGACGGCGTGTATGACCGTTTTGCGGAAAAATTGCAGCAGGCAATGAGC  
AAACTGCACATCGGCGACGGGCTGGATAACGGCGTCACCATCGGGCCGCT  
GATCGATGAAAAATCGGTATCAAAAGTGAAGAGCATATTGCCGATGCGC

Contig 32 (450 bp)

GGTGGATGCTGGCGATAGCGTCATCCTCGCTTATGCCGTGCAGCGGGCAA  
GGATAAAGCGCGGATAAACATGACCCGGCATCAGCCCCATGCCCGCAGA  
GTACGGATTACCTTGCCGGTCAGCGCCAGCGTGAATGCGTGCGCCCGT  
GATACGCGCCGCTAAAAGCGATGGTGCCGCTACGTTTGGTGGCGGCGCGG  
GCGATTTTTACCGCGTTTTTCCACCGCTTCGGAACCGGTCGTAACCAGCAG  
CGTTTTCTTGGCGAAATCGCCCGGCACCTTCTGATTCAATCTCGCACA  
GCTCCAGATACGGCTCGTAAGCCAGCACCTGGAAGCAGGTGTGCGACAGT  
TTTTTCAACTGCGCTTCCACCGCGGCCACCACTTCGGATGCAAGTGCCC  
GGTATTGAGCACCGTAATCCCGCCGCGAAATCAAGATACTCACGGCCTT

Contig 33 (500 bp)

ACGTGAGGTTTGGGGGAGGAAAGCGGGGACGAGCAGCCCCAGAGGAGTG  
GGGGCTGGCCTGTGGCTGATGAAACTCTGAGAAGGTTAAGAGCCCCATT  
TTTGTCTTCTCTTTTTTATTATGGAATTTCAAATGGATGCAAAAGTC  
CCAAACCTAACTGGACATCTTCTTGGTACCAGGAACGGTCAGGCACCTAT  
GATGCACCGAGCCCCGAGGGAAAAACCTGCCGTCTGGAGCCACGGTC  
CAGCAGGGCACACAGGCCCCAGCCCGCAAGCGGCACGGCTGAGTCAGTGA  
ATGGCGTGCCCTCTGGTCAAGGACGGGCACTCTGGACCCAGGGAAGCCT  
CTGAGGAGCCCCCTTACAGCGTCAAAACTGTTAACAGGGCCATGTTTCG  
CACCCCCCACACAGTGGTTCAGAAGCAGACCCAGGCATCGTAATATG  
TCATCCGTGAGTTCCCTGTGTGCCACCAACAGAAAGCCCATCGTCACGTT

Contig 34 (400 bp)

CGGCATCGATGTACATGGTACGCAAGGCACTCGTAAGGCCCGAGCCTCT  
AGGCCTTGTCAATTGTACCTGCTGCTCGCGGGGATCAGCAGCCAGGCTTG  
TGACCCCGGCCACTTTGACAGATAAGGACACAGAGAGGCCACAGCACTGG  
TGTGAGGCCCCACAGCCAGCAGCCAGGGCAGGGAGGACTGGGTCTCACC  
TGCTCAGCTGGGCCAGCCTCCCTGGGAGTCCCGGAGTCTCCCCAGCTT  
AGGAGTGTCCCTGGAACCTCTTCTCTCCCTTCCCGCCCTCACCCGGAC  
CCCTGCTCCCCCCCCACCAACCCCTCCCCCTCTTCTTACCTTGAG  
CTCCCCCTCTGAGGACCTCTACTGTTCTGCTTATCCTCCCTTTGAGCCA

Contig 35 (500 bp)

TGGCGGTGAACTATGTCGTGCGTGAAGAGCATTTGTGGTGGTAGCGCGT

FIGURE 6, CONTD.

TATATGCGGGAAGTTTAGGCGAACTGGACAGCCTGGGTTTATCCGGTAGC  
GAAATCCGCTTTCACGGTAAAACGCTGCTAGCGCTGGTGGAAAAAGCGCA  
GACATTGCCGGAAGATGCCTTACCGCAGCCGATGCTTAACCTGATGGACA  
TGCCGGGTATTCGTAAAGCGTTTAAAGCGATTAAGTCGCTGATTACTGAC  
GTGAGCGAAACGCATAAGATCAGCGCCGAATTGCTGGCATCGCGTCGGCA  
AATCAACCAACTGCTGAACTGGCACTGGAACTGAAACCGCAGAACAAATT  
TGCCGGAGCTGATTTCCGAGCTGGCGTGGTGAGCTGATGGCGGAAGCATT  
ACACAATTTATTGCAGGAATATCCGCAGTAAAAATCTTCCGAAGCCGGACT  
GGGCGCGCTCAGCGCCACATCCGGCTTCGGCAAACCTACAAATCCAACACC  
Contig 36 (500 bp)  
GATTTCACAAGCCTGACCCACGCGGAAATGCGCTAACAGCGTAAAGTCGT  
GCGGCCAGAATTTTTTCGTCTCTTCGCTTTCGCTCAATTCAAAAGTCAGC  
GCTACGCCATCAGCATCTTCATGATGTGATTTACGCGTCCACGGCAGGTT  
GCGGGCAAAACCGTGCGCAGGCAGACCTTGTGTGCGCGCCGACCAAAACC  
ACGGCCAGCAAACCGGTACGCCACCGCGAATAGCGACGCCATTTTGAAC  
GGTGTTGTTGTTGCTCAACCCACAGAACTTCTTCTCACCCGCGAGTTTCCA  
CGAGAGAAGGTGTGCGCCCTGTAATGCAAAAGAGGCTTTTACCTGGGGAT  
GATCGACCACAATGAGGTCCAGTTCATCCAGTTTACGACGGGAGAGGACA  
GGGGAGATTTGTTTCGATGACCGGAAGGGCAAAAATTTTCTTAATCATGAC  
GCAGTCTTTAACTTCATTTTATCAGGTAAAAAAGAGCGACCGAAGTC  
Contig 37 (300 bp)  
ACCTGATCAGGCTCTGCACTGTGTTTCATCAGCGGAGCCGAGATATTTGAC  
CGCCCCATGCATAACGGAAGGCGTGGGTAAACCCCGGGCGCGTTCCTT  
TATCAAGATGACGTTTCAATATTTCCGGCAGGTGCAGTTTGTATTCCAG  
AAAGCGGTTGAGCGCGTATGAATATAATTCTGTGGGATTTGAAGCATCCT  
TTTCCCTCCTTCGGTGAATGCGCTGAAACCGGCTTATCCAGCCGGTTCA  
GGGTACGCCCTGATAATTGCAATTTAAATACCATTTATTGGGTACTTTTT  
Contig 38 (450 bp)  
ATCCTTTTGGGGTCTGGCAATTACGCAATAAAGAAGGCCCCCATGCGATT  
AAAGTCACCGGCCCCACTGTCGTCTAATCATGGAGAAATTGTCATCAGTG  
GGGTCTCGATGGGCAGGGGATTGCTCTGCGTTTCTGGTGGGATGTTAGCG  
AAAACATTGCCAGTGGTTCATTTAGTGCAAGTGCTACCGGAATATTACCAG  
CCAGCGAACGTCGTGTCGCTTTATGTTTCAAGGCTGGCGACGTCAGCGAA  
AGTGCGGATAACGGTAGAGTTTTTACGCCAGTATTTTGCCGAGCACTACC  
GGAATGTTTTCAGTGTGTCATGCCTGATTATGATTCAATTATCGGGTTGA  
TATCAGTTTTAAACCTGATTTTCTCCTTTCTAAGCCGCTACAGATTTGGT  
AGCATATTCACCTTTAATCGCGCATGATCTAAAGATAATTGAAGAGGTTA  
Contig 39 (450 bp)  
AATGTACTGGCAAAAAGCCAATGGCGAAGCGTGGGGAACGTTACATGCTC  
TGCTGGCGGATATTAATAGTCAGGGTCAGGTGCAGATGGCGATGAACGGC  
GGCATCTATGATGAAAGCTATGCGCCGCTCGGTTTGTACATCGAAAACGG  
TCAGCAGAAGGTGGCGTTAAATCTCGCTTCAGGTGAAGGGAATTTCTTTA  
TCCGTCTGGCGGCGTGTTTTATGTCGCGGGAGATAAAGTCGGCATCGTT  
CCTCTGGATGCCTTCAAAACAGTAAAGAGATTTCAGTTTTCGGGTGCAGTC  
AGGGCCAATGTTGATGGAAAACGGTGAATTAATCCGCGTATTCATCCCA  
ACGTGCGCTCAAGCAAAATTCGTAACGGTGGTTGGGATTAATAAACATGG  
GAACGCCGTGTTTTTGTGAGCCAGCAGGCAACAAATTTTATGATTTTG  
Contig 40 (400 bp)  
GACATTAATCATTTCAAATCAAAGCCCCGTTTTTCCATCGCCGTTTTGG  
TGGCGTGGCACTGAACGCAATCGTTACGAGTGTAATAGTAATGCGCATG  
ATTCGTATTTCCGTTTAAATGAAGATACGGCGCGATGATACGCGTCGGG  
TTGTCTCTCTGTTGATACAGAGATACTAGATGTAGTTGAAAAAGATTCA  
ACCACACAATATATAGCCAGTAGGGGTCGAAATTACCCTGGATATGAGC  
GTGACGGGGTAGGGGATTTTGTGATTCACCAGGCAAAAAGAAACCCCG  
AAGACAGGCTTCGGGTCAAAGACGCGTATTTATTATCATTTTTGCACTA  
CGATTTGCGCATGCTTAACAGTGCGCCGATTAATATCTACCGCAGCTG  
Contig 41 (500 bp)  
GCAAAATCACGTCCGCGACCTGGCGTTGTGCTGGGCCATATTGGCAAAG  
GAGCTGGATTGCGGTGCCTGCAAAGTGCCCTGAATAATGCCATTGTCCTG  
TACCGGGAAGAAACCTTTTCGGAATGAACACCCACAGCAGCAGCCTAAGCA  
GCAGCGTGCTGAGTGCCACGCTTAAGGTACGCCACGGATGATTTCAGCACT  
TTCGCCAGTCCACGACCATAGGCGGCGATTATCCTGTGCAACATTTTTTC  
CGAGGCACGGGAGAAGCGGTTCTGTTACGCAACGACTCCTGGCTGAGCA  
TCCGCGCGCACATCATCGGTGTGAGGTCAGCGACACCACCGCTGAGATC

FIGURE 6, CONTD.

AAAATCGCTACCGCCAGGGTAATAGCAAATTCGCGGAACAGTCGCCCCGAC  
GATATCGCCCATAAACAGCAGTGGGATCAACACCGCAATCAGTGAGAAGG  
TCAGCGAGATAATGGTAAAGCCGATTCACCTGCGCCCTTGAGCGCCGCC  
Contig 42 (400 bp)  
AGCTATCTACGGCAAAAGGCACGGTAGTCAATTTCTGTTGTTAAATACATC  
AAGCGTTTGGCGCCGAAATACCATCTGCCAGATGCCATTTTCAATTCGTAG  
CGCACTGCATAACGGCTACCGGATGCAGTACGTCAAACCCGAACCTGGGGC  
CGGAAGGATTTAGCTTTTCTGCAATACACCGCGGCACCACTGGTGTGGC  
GAAAGGCGCGATGCTGACTACCGCAATATGCTGGCGAACCTGGAACAGG  
TTAACGCGACCTATGGTCCGCTGTTGCATCCGGGCAAGAGCTGGTGGTG  
ACGGCGCTGCCGCTGTATCACATTTTGCCCTGACCATTAAGTGCCTGCT  
GTTTATCGAATGGGTGGGCAGAACCTGCTTATCACTAACCCGCGCGGATA  
Contig 43 (450 bp)  
GATTAGCGCCAGATGCTCGCCATCGAAAAGTTGAATCAACCCAGCTGCG  
GGTAATAAGTGCCGTACGAACAAATTCAGTATCCAGGGCTATCGCCGGA  
AAGGCACGGACGGCTTCACACAAAGAGCCAGCGCATCGTCCGTGGTAAT  
CATTTGGTAATCAAATTTGTTTTCTTTAGTGGGCGTCAAAAAAACGC  
CGGATTAACCGCGCTCTGACGACTGACTTAACGCTCAGGCTTTATTGTCC  
ACTTTGCCGCGCGCTTCGTACGTAATTTCTGTCGCAAAATTTTTCCGAC  
GTTAGATTTCTGTAATCATCACGAACTCCACCAGCTTCGGTACTTTGT  
ATCCCGTGAGCTGACGGCGGCAAAAGTCACCAGTACTCTTCGGTAAGC  
GATGGATCTTTTTCACTACGAAGATTTTCACCGCTTCACCACTGGAGCC  
Contig 44 (750 bp)  
GAGCAGCCCGCGTGATGACAGGCATGCGCCCGCGTGGGCTCTCTCTCTCT  
GGTGCCTGAGTACAGGATGGCGCGGTGGGCGCGGTGGTGGAAGCGGT  
CCTGGAGGGCTCGGGAGGGAGGATGCGCTCAAGCTGGCTCCCCGTGGGGC  
TGGCCCGGAGTAGCCTCCGTGAGGGCACCGTGTCTGCTCCAGAGCCCCG  
TCCCCGGCTGCCCTGCCCTCCCTTCCCTGCCCCAGTTCCCCCGGAGCCCC  
TGGATCCCGATGGGAGGCGCCCTTGGGGAGAGGGGACCAAGGAGGGGGCC  
AGAGCTCTGAGGCCACCAAGACCTGGCCAGGACCCCTTCGTGGGAAGAAGAG  
GTGGGGCCCCAAAGGCACCTAGAGAGAGGGAGGCTCTGCTGGCTGGGGGGC  
CTTCCAGGCGGGGCTTCCAGGACGGGCCAGTGTCTGGGGGCTGGAGGGA  
GTCCCTGGCTGCTGGGGGGCGGCAGGAGCACCTGGGGCGTCTGGGAAGAG  
AGCGGGAGGAGACTGGAGCCAACTGGGGGGACAGAGGAGGGGTCCAACCC  
CAGCGGTGGTGTGGGGGTGCTGGTGGTGGAGGCCCTGAGAGGCTGTGCT  
GGGGGGCAGAGCGGGTGTGGGAGGGGAGAAGGGTCCCCAGGGCTCATG  
GGCCCTTCGAGCAGTGGCAGTTGGGGTGGGTGGCTGTCTCTAGGGCTGT  
ACCACGGTGGGTGCTGGAGAAAGAGGTCTACCCCTAGTCTTTGCTGCA  
Contig 45 (300 bp)  
TGGGGACCCCACTCCAGCCCCACTGAGTGACGCGCCCCCTGTGGTCCCA  
CCGCCAACCTGCCTCACACCAGAGGGGCTGTGGCCACACCTTGTCACA  
GCCTGTCCCTGAGACCACGAGCCCCGGGCTCAGCCCCCTCCTACCCCT  
GGACCGAGGAGAAGCCCCACCTGGGCTCAGCTCTTGGAGCTAAACTTCC  
AGGAAGTTCTGGTGCCCTCGGGTCTTAGAGCATGGTGGGAGGGGGATG  
CTGGTGGGGCGCAAGCCCTCCCCACATTCGCACTCGACCCGGTGGNG  
Contig 46 (300 bp)  
CCGGCTAGAAGCCACGAGAGCCCCAGGCCCGCCCGACGTCTCTCTGCTG  
AGGGATTGCGCAGCCCTGGGGCCACAGGGCTGAGCAGACCTTGGGGTTC  
CGGTGTGACTCCAGCCAGGGTCCCTACTGTGTAGGACACAGGGCAGAGTC  
AGCCCTGGGACCATGGCCACAGCTGCTCCCGCTGAGCCGGGCCCCCGC  
CCAGGCTGGGGCCCCCTCAGTGCACTGTCCCAAGCCAGCTGCTCTCCCCAC  
CTCCACCTTCTCCATCCAGGTCTGCCCCACGGCCTTTGCTCAGGCCAG  
Contig 47 (500 bp)  
TTGACTGCACTAGCACGAGCTCTGTACCCGGGGATCTGGGCTCGGGAGA  
AGGGAGACCCCCACCCGGCAGGCGGAGGGCGCTGTACACCATGACTCT  
CAGCCTTCCCCACCCGACGACAGAGTGAACCTCTCCCAAGCCCCCACT  
CACCCAGGACCGCACACCCCGTGAATCCTGCGAGTGGGGGCGGCTCAGGG  
GCCCCGAGTCCCAAAGGAGTCTGCTGGCCCTGGGGGGGAGGGGAAGCAGC  
AGGGTGGTCAAGGGTCTCCCTGGTTGGCAGGACCACAAGCTCAGCCCGCT  
GCCTCCAGAGGGCAGCCGACACCAACCAAGTCCGGGGACCCACGTACC  
TCAGCTGCTGCAGGTGCCCTGCCTGTACTGGTGCCAATGGGGCCGCTGG  
GTGCTCCCATGGACAGCTCGCCACTCATCCAGCCGCTACCCCOCTTCC  
GGGTCCAGTGTCCGGCCGGCCACCCGCTGCCAGCCCTGGCCTCCTCTC  
Contig 48 (500 bp)

FIGURE 6, CONTD.

GGGGTTGCCGAGGCTGCTGTGTAGGTGCGAGACGACGCTTGATCTGGC  
GTGGCTGTGGCTGTGGCTGTGGCTGTGGCATAGGTGAGCCACTGCGACTC  
CGATTTGACCCCCAGCCCGGCACTCCCACATGGCACAGGTGCAGCAGGG  
AAAATAAATAAATGAAATAAAAAATAGGTGAAGACAGTGGATTTTCATCTCT  
TGGGGTTGCGGTAAGCTCTACACAATAGGGAGTTTACCATTTTACCTGTT  
TCAAGTGGCACTGAGTCAGCTCAGTCTGAGGGCCACAGATGCCGTC  
TGCCTGGGAGATTGTTCTCTCACCACACTGCCCTCTGTCCCCACTAAA  
TACTCACTGCCCTCCCCGTCCCAAGGGCCCCCTGCCCCACCTCTGCTTCC  
TGTCTCTGAACTTGTGTGGCCACCAGCGACCGTCTGGTGACCTCACTCTTC  
GGCCCCATTTGTCGCACACCCACCTGGCCTCTCCCCGGCATGGGCAGAN

Contig 49 (600 bp)

GGGATATTTGGGGCATATTTGGGGGGGAGATCCCCACAAGGCATTTGGG  
GTTTGTGGTTTGAATGCCCCGGGCGCGATGGAGGGGCGGGGAAGAA  
TCTAAGCCTTACTTGGGGAGGGTTGGGCCCCGGGGCCCCGGGCGGAAAT  
GCCCCCAAGACAGAAGGTGTACAAAATTTCTCAAAAGGGTGACCCCTAAT  
GAAACGGGTCCCGGTTGGAAGAGGTCAACAGGGTGGATTGGTGGCACCG  
CAGAATTTACGACATTTTGGCTCTCTTCCAATGGCCGGACGCTTGGGGAT  
AGGCGCCCCCGTGGACGGCGGGGTCTCGGGTGGGACGGGCGGTGAGGGGT  
CGGTGACGCTTGGCCTCTCTGACCGCTCCAGCTCCTTGGCGAGCGTGCG  
AGCGCGGGGGCGCGCAGGAGGGCGCGCAGGCCCTGCGCAGGCGTTGG  
GCGGACTGCTTCCAGGTGTATAGCGGAAGAACTTGCCACGGGGTATCT  
GGGGAAGTTGTCTGAGAGGGGAAGGGCCCGTCAGGGGGGGGCTGGCCCC  
CCAGCCCCGTGTCCAGAACACAACTTTGCGGGGTCTCTGCTGCTGCC

Contig 50 (179 bp)

ATCTTCATATTCATGCAGAAGACACTCTCCTGCCTTTCTATCTTGGGGAA  
AAGGACGATGTCACTTATGCAATAAAGCCCACTTGCTGGCCGGGGCTTGA  
CATTATTCCTTCTGTCTGGCTCTGCACCGTATTGAACTGAGTTAATGG  
GCAAAATTTGATGAAGGTAAACTGCCACC

Contig 51 (500 bp)

CTCGGGCTGCTTCCAGGGGGCTTGGGGAGCCATAGAATGCTATGGAGCA  
AGAGAGTGCTATGGTCAGACGACTTTGGGGGAAGGTCTGGGAGAAGAGGG  
GTGACTGGCCACTGTGATAAAGAGTGGGCGCTTCTTGAGATAACACGGT  
GGGCAGCCGAGGTGGACCTGTGCAGGTGGAGAAGGCCTCTGCCGCGGGC  
AGTACGTGGCTCTGGGCTGCGGACACGAGAAAGCCACCTCCACGGCTG  
CCTCCAGGCGGGCCTTCTCTTCCACCGCGGGCCATGCCAGGTGC  
AGGTGCCATCAGAGGTGCTCAAGAGAAGCTCTGGGCTGGGGTTGTCCCA  
GTTCCCGGAAGCCCCGTGTCCAGGGGCCACCTGAGGAAGCGTGGGCGCA  
CAGAGACTGTCCCTCGGTGCTCAGAGAGGGTCCCGTCCACGGCAACGA  
CGCCCAAGGCGGAGGTGGTCAGAGGTCTTGGGAGGGAGGATGGCCGCGCA

Contig 52 (900 bp)

TGTGTTGCACCTGTTGCTGCCTGTGACTCTAGAGGATCAATACTCCTTA  
CATAATTAAGGAGAACAAATGGAATTAATAAATTTGATGGGACATATTT  
CTATTATCCCCGATTACAGACAAGCCTGGAATGGAACATAAGTTATCG  
GATATTCTACTGTTGACTATTTGTGCCGTTATTTCTGGTGCAAGGGCTG  
GGAAGATATAGAGGATTTTGGGGAACACATCCCGATTTTGAAGCAAT  
ATGGTGATTTTGAATGTTTCTGTTTACGACACCATTTGCCAGAGTT  
GTATCCTGTATCAGTCTGCAAAATTTACGAGTGCTTTATTAAGTGGAT  
GCGTGACTGCCATTCTTCAAGATGATAAAGACGTCATTGCAATTGATGGAA  
AAACGCTCCGGCATTCTTATGATAAGAGTCGCCGCGAGGGGAGCGATTCT  
GTCATTAGTGGCTCTCAACAATGCACAGTCTGGTCATCGGACAGATCAA  
GACGGATGAGAAATCTAATGAGATTACAGCTATCCAGAACTTCTTAACA  
TGCTGGATATTAAGGAAAAATCATCACAAGTATGCGATGGGTTGCCAG  
AAAGATATTGCAGAGAAGATACAAAAACAGGGAGGTGATTATTTATTCGC  
TGTAAGGAAAGGAGGGGCGGCTAAATAAAGCCTTTGAGGAAAAATTC  
CGCTGAAAGAAATTAATAATCCAGCGCATGACAGTTACGCAATGAGTGAA  
AAGAGTCACGGCAGAGAAGAAATCCGTCTTCATATTGTTTGGCATGTCCC  
TGATGAACTTATTGATTTACGTTTGAATAGAAAGGGCTGAAGAAATAT  
GCGTGGCAGTCTCCTTTCCGTCCATAATAGCAGAACAAAGAAAGAGCTC

Contig 53 (450 bp)

CCAGCCACGAGCTGGACCTCCCGGAGAGGGGCTGCCTCCTCTTTCCCGC  
CCAGACGCCCCCAGCAATCTGTGGCCAAGAGGGAGTGATACCGAAGATG  
GCCACATGGGGGCGCCAGCCACAGGGAACCCAGGAAGGCGCTGGACCG  
TCAGGAGTCAGGGCTGCTGTGACCCATGTGGCCTGGGGACTTTCCACAG  
CCTGGTGGAGATGGCCGGGCACACCGCTGCCTCGGGGAACGTGCACACG



FIGURE 6, CONTD.

GGTGGTACATGTGGCCGAGCCAGGGCACAGGGTGAGGGGAGAAGGGAG  
CATGCGGGTGCAGACTCGGAGCCCGCGGTGAGGTGCTGGGTCTCAGGA  
CACGCTCTGGGAGTGGAGGACCCCCATCCAGCCCTCAGGAGTGTGTGC  
CCGCTGCTCCCCGGAACCTCACAGACACGAGGGCACACCCAGCCCC  
Contig 54 (1133 bp)

ATGGCGCTCATTAGAATTCGACCTCGGTACCTTGGGATCTTTTGACCCCT  
ACCTCACGCCATCTACAACATTTACCTCCGAATGAATGAGAGACACAAA  
AGCAAATTCATAGAAGAGAAAAAAGGTAACCTGGACTTTAAAAATGTAA  
ACTTCTGCTCTTTAAAAGGCAGTGCTAATGAAGTTCAAATACAAACCACA  
GACCATAAGAAAATACTTGCAAATCTTGTCTGACAAAGACTAGTGTTC  
GAACATACGACGATCAGGGAGAGGAAAAACAGCAATCTATAAACTGGA  
CAAAGAATTGGGGGGAAAAAAACCCACTTGGCCAAGAAGTTGGTAAATA  
AGGCCATGAAAACATGCTCAACATCATGAGTCATTAGAAAAATGCAAAAT  
AAAATTATAATGAGATACTACTACACAGCTATTTGAATGGATAAAAAATG  
TTTTAAAACTGATTATACCCAGGTTTGGCAAGAACATGAGAAACGAGAT  
TTTCACACACGATTGGTGGAAAAACAGAAATGGTCCACCCACTTTGGAAA  
AGAGCTGGGCACTTCCCTCAAAGTTAAACATACATCCAGGACCTCACAC  
AGGCTTTCCACCACAGGTGTTTATTCAGAGACATGAAAGCGCTCATCCA  
CAGCAAGACTCGTAAATGAAGTTTATAGCACCGTTTGTGGCCCGAAGT  
AGAAAACCCAAATGACCTTTAACCAGAGAATATCTAAACAAAATATCCAT  
TCACATTAATCACCCATAAGAAGGAACGGGCTATGGGGACGGGAACCGTA  
TTGAAGAGGGTCAAATACATACGACGATCAAAGAAGCTGCCCAAAGG  
ACACACACTGCAGGGTTCCATGGACTGAACTCGAGAAGGTGAAACTCG  
CCAGCAGTGACAGAGAGCAGGTCCGAGATCAACCTGATGTGGAGGAAAGT  
GAACCCCTCGTGCGTTGTTGGCAGGACTATAAACTGGAGCAGCCCTACGG  
ACAACAGTAGCCCGGGCTCCTCTCCTCCATCTCCCTGGGGAGCCTGAGCC  
TTGAGACGCTGGGGCAAGTGACGGCATGCTGCCTCACGTGGGGCCCCGG  
TGAAAACACCTGGCAGCTGGGGAAAGAATCGTA

Contig 55 (735 bp)

TACTGCCTGTCTCTATGGACTTGACTCCTCTCGGGACTTCATGCGAGGGA  
TCTTACAGAAATTTGTCTTTTGCATCTGGCTTGTTCCTGAGCATCGTG  
TCCCCAAGGTCCATCCATGTTGCAGCCTGTGTGAGGATTTCTTCTCTTT  
CAAGGCTGAATAGTACTCCACTCTGCGGATGGACCACGTTTGTATATCC  
ATACTAGTAAATCCATACTAATAACTTGTTCCTGAAGCCACAGCTTAT  
GCTACCTTCCGTGGGCTCCTCCTGCGCTGTCTACGCCTTCTGCTATA  
GCCCCATCCCCCTCTCATCCAGGCCACGCCTCCTGTCCCCTGGACACTGTC  
CCAGAAGCCAACCTGCCCTCTGACTGCTGCTCTGCGTGACGGAGGACAAG  
GCAGGCTCAGGGGTCCACGGGCTGGGGCCCCAGGGCTCCCCATGGCTGGT  
GCCCCCTTCTGATTCCAGAAGTACAGTGGCAGCACCAGCTTTCCAGCTGC  
CCACCTTCTGTCCGACGGCTGCTCGGGTGGGGGCAGGTGGGCAGTGATG  
TCACCTGCTGTAACCACCTACCGTCTGCTCATCCCTGTCCAGGAGGTAC  
GGTGACCTTGGCAAACATTCTGAACAACACACACCTCCCTCTGCTTAGAG  
GCCGGGGGCTCCCCGGGTGACTGGGGGCACAGGCTGACCCAGCCTGTC  
TCTGTTCTCTGAAGGACATGATAAGTACTGCAACA

Contig 56 (500 bp)

AGGAAGAACAGGAAACAACGGGGTTGAGGAGAAGAAACGGGTGTCTGGCA  
GGGGCACGTGCCAACGGTCCACGGGTGCTGCCGCGCTGCGGCTGGCGC  
CAGAGGGGGCAGCTCCGCCCTCGGGCCGCGCCCTGCCGCTTGCTGGC  
TCGCGGCTGGGCTCTGCTTGGCTGGGTACAGCTGGGTGCAGCCGAGGC  
TGTGGTGGGTGCCCGGGTCCAGCCAGCCCGGCCACCCGGCCCGTCTC  
GCCGGCTTGGCCCGGGCAGCCCTCCTGCACTCGAGGAGTCCGCTGACGG  
GCTGATTGGTCCACAGCCTCAGATGCAAACAGCCCCACGTGCCTGGAGC  
CAGCCAGCCCGGACACCTTGGTGGAGGCAGGAAGGCAGCAGCCTGGAGA  
GCCGCGCCGGATGATGCTGCGGGGAAACGGGGCTCCCGCCGGGGCGCCC  
TGGCTCTGGCCAGGCTTGGCTTGAATGCTGACGTGAGCGGTGGCCCTATA

Contig 57 (500 bp)

TGGCGTTGCACTGGCTCTGGCGGAGGCCGGCGGCTACAGCTCCGATTGGA  
CCCCTAGGCTGGGAACCTCCATAAGCTGTGGGTGCAGCCCTAAAAAGCAA  
AAAACCCCAACATATATATATATATATATATAATTATGTTAAATACA  
CATAAAATAGAATTTACCTTCTTAATAATTTTCAGTGCACAATTCAGTGG  
CACTAAGCATTTCATGCGGCCGTGTCACTGCTCCAGAACTTTCCATCT  
ACCCAAACGAGACTCTCCGCCCATGGAACACGCCCTTCCCTTCCCCG  
GCCCTGCCCGCCAGCTCCTCCTGTGTCTGTGGATCCGGCTCCTCCAGG

FIGURE 6, CONTD.

GACCCCGTGCCTGGGCTCACAGAGTGTGTGCCCTCTGTGACCGATCGTC  
GTGTCCCCGAGGCCCGTTCTGTGGCAGCTGCGTTATGACCGACTACCTTC  
GAATGCTCAGTGACTGCCGTGCATTGGACACGCAGTCCGCTACCTTTTTT  
Contig 58 (550 bp)  
TGCTTTCTGTGCCCCCTCCAGCTTGGGACCCCAGCAGGGCAAGGGGTGT  
ATAGGGCTTAAGGAGGCAGGGGGCGTCTCCTCCCGCTGGCTGCCAGAGC  
ACCCCCAGCCCCCGCTGCCCTCGTCCATCTCCAGCCTGTCTTTCTGT  
GCCCTCCCTGTCCCGGGCGGGCCGACACTGGCTTCCACCTCCCCACCCA  
ACTGGCGGCCCGGTCTTCTGTGTGAGGCACCCGAGGTCCCGCTGTGT  
GGGACCAGCTGGCAGGTGGGTCCCAGTGTCTTCTCAGCGTGGGGCTTTGGA  
GGGGGGATCTGCACATACCATCCCTTCAGGCCCGTGGGGAGCCTGGGGA  
CCATCCGGGACCCCTGTGGGCAGGCCAGAGGACTGCCAGGAAGAGACC  
AGGGGACCAGGCAGTCCCAGGCCCTCAGCTTCAGGCCAGGGGAGCCCA  
CCCCCAGGTGGCAGGTGAAGCCAGGCCCAACCCACAAAAGTGGCCGCA  
GGGAAGTAGGAGGACAGGAGGAGGGGAGGCCAGGCCCGGGCCGCTTG  
Contig 59 (800 bp)  
TGAGGACGCGAGGCCAGGCCTGAGTGTGCCAGCTTACACCCCTGGCAG  
CTTCGTCCCTCCTGCCCTAACCCCATCCTACCCAGCAGCAGGGGCTC  
CCCCGGTGGGGCTTGGTGAGCGTCTGACTGGGGTTTGGAGTCAGGTCTGC  
TCCAGGCTCAGCCCCATCCCCAAGGGTGGCTGCAGCACTGCTGCCAC  
CCCTAGCGCCCCCAGACCTTCGCCCTCCAGCCTGGATGTACCCACGGA  
CCCTGAAAAGTGGGGCTGAGCAGGTGCCCTGGCTGGAGTCCCCCTGACTT  
GGGGCTGGCCAGGCTGCCCTGGAGGGGCTGTGGGGGCACAGCCTGCCCA  
GGGGCCCGCTGGGCACTGGCTCTGGAGCTGACGACAGGCAGGCCCTCTCT  
TCTTGGCGGGGCCACACCTGCCCTGGGGTTTGGGGCAAGGCGGGCAG  
CCCCATGTACAGCGGGGGCGAACCAGGTAATTACAGCCTGGCAGCCCGCT  
CCCCAGACCCCCAGCCCCGAGGGCCCCCAGGCTGTGCCACCAAGA  
CTTGGCATCCAGGGCCCCAAGCAGGTCAAGGGCAGCTGCTACAGATTCTT  
TTAAGTTGAGACAGAATCGACACATGACAAGTTCCCTGGTTTATAGTACTT  
CGCTGCCGGGGCCGCGCAGTCACTTATGAGCCAGCACACCCACACAGG  
TACAATTGCTCTTCTCAAAGAGGGCCCTGAGAGAGCGCCTGTCTTGGCT  
CAGGGGTAATGAGCCCAATGGGTATCCATGAGGTTGCGGGTTCATCCCC  
GGCCTCGCCGCTTGGTTA  
Contig 60 (500 bp)  
GGCTCAGGAAGCGCAGGGGAGCGTGTGGGGCGACGGGAACCATGGGGGT  
CTGTCTTCCCGCTCTCCTCAAGCCACCGCCCTGCTGCCACCTCCGAC  
TCTGCAGCCAGCATGCCGGCTAGAGCCCTGTGCACCCAGCTGGTGGCCT  
CTGGCTAAGGGCAGTGTGGCTGTGGACGCGTGTCCCTCCCCAGCAGCC  
CAAGGGTCCCCTGTCAGGCTGGTGGCTGAGGTCTGCCCTGTGTGGTCC  
TTGCAAAAACCCGCCCTCTCCTGCCCTTGAGGCGTGAGGGAGACGCGG  
GCTGGGCGGATGCCCTCGGGCACAGCCCGCGCGGTGGCGCCCTGTGAG  
GAGGGGGCTCCGACGTGCCCTGACGGCCCTGGCCGGGCGGAGAGGGTGAG  
GCCACCTCCTGGCCACGTCCACCCAGCTGCCACGCCGCTAGCCAGTGGC  
CCGGGGCCAAGTCAGCAGAGCCAGGCTTCCGACAAGCAGAGGCTGTAGGC  
Contig 61 (700 bp)  
GATGAGGAAGCCGCTGCTCGTGTGCTCGTCTTCTTGGCCTTGGCCTCGT  
GCTGCTATGCTGCTTACCGCCCCAGTGAGACTCTGTGCGGCGGGGAGCTG  
GTGGACACCCTCAGTTTGTCTGCGGGGACCGCGGCTTCTACTTCAGTAA  
GTAGCTCAGCGGGGCACGGGGGCGGGGCGGACACAGCAGGTGCTCCATCG  
GTGCTGCCCCGCTACCTGTGCGGGTCTTTCGGATGATGATGGTGTGGGGGA  
CGGGGGGCGGGGGCGGCCAAGGGAGGACCTCTCCTCCGAGGGTCTGAGA  
CTTCAGACCGGGGGCGCCCTGGCCGTGCGCATTGATTGGCACCTGCCATG  
TGCCTGGCTGGGGCTCACACCCCTGACGTTCTTGCAGCGTGAATCGAAA  
CGGGAAACCGAAGGGACGGGTGGCACGGGGTGGGGAGGCAGACCGTGAGT  
GGCAGGCGTGGCAGGGGTTCTTTCGGGCGGGGTGGCCAGGCAGGCCCCA  
CAGGATGACAGCCTGTCCCCTCCTGCTCCTCCTTGACCTGCCACAGCCA  
GGGCTGCAGGCACTGACATTACCCATGGTATTGTGGTGCCTTGACGTCT  
TGGCAGTGGGCATTGGGTTTATGGACTGTTTGGATTGAAAAGTGGGAATA  
AGATGGGGTTTGA AAAACCAATTAAGAAATAAAAAGGCGCCCTGTGGGC  
Contig 62 (300 bp)  
TTTGAAAAATTTTGTAGTCAGTGCAGAAATTCGCATCTATTCCGCATTTCAGG  
CTTCTCTGTTTTCACCTTGCCCTAGTGCGGATCTTCTATAACCACCACAG  
TGACGTTTTCAAGGTACTTTATTGAATAATAAGAAAAAGTGCACACAAT  
CATGTAGTTAACTTTCTGTGCTCTTGGCAGTTTGAAGGGACCTCTTTT

TTTCTTTTTAGGGCTTCGCGGACGGAAGTTCCCGGGCTAGGGGTTGACT  
CAGAGCTGCAGCTGCTGGCCTACAGCACAGCTCTTGGCGGCGATGGATCC  
Contig 63 (450 bp)

TCCTGGGCCACAGGCTGCAGCAGCTCACCTGGGGGCTGGGGTCTCGCTCT  
GCGGATGGACCCATGAAGGCCGAGCCAGGTGGGGGCCGAGACGGCAGGG  
CAAAGGGTCTGCACACACAGCGTCCCCCGACCCGGCTTCTCTGGGTCT  
TGGGGGGTTGGCGAGGCTTCTCTCAGTCTGGGTTTCTGGGGAACCTTCA  
AGAAGTGGGAAGTCTTCCAGAAAGTTGGGGTGAGGGGAGGTACCCCCAAA  
GTGCTGCTCCTGTCCCCATCCCCACCCCGCTGCCATCGGCGAGACCCC  
GGACCGCGTCTCCCTGCCGAGGTGTGGGGTCCCCCCTCTGCCGGCCAG  
GCTGGGCAGGGGTGAGCGCCCCCTGCTCTGCACTCGGGACTCAGCCTGGG  
GAAGCGGGCCCCAGGAGGTCTGGCCTGGACGCGAGTGACCTTCCACCG  
Contig 64 (500 bp)

TGTGCATCAACCCAGTGGCCACGGGGGTGACCCTCGGCCGGTACGCC  
GCCCGCTCTCCACGGAACCGGGCCTTGGCCTGAGGCAGAAGGACCCAG  
GACTCCATCCCTGCCCCGACTCTGCCGAGGGTGCGGTCTGCACAGAGA  
CCCTGTGGGGGTGAGGCCGCTCGGGGCTGGGGTTGAGATGGGATGGTCAG  
GGCGGGCCCCGCGGGCCTGCAGGAGGCTGGGTGAAGGAGGGGGCCAGCT  
CAGACGCCCCAAACCTAGCTTGGGAGAGCTGCAGCCCCGCCCCGTCAAT  
CGCGACAGCCTGCCACAGAGGCATTCAAATGAGAGACAAATATTTGGG  
CTTGAAGACTATACCCAGCCACGTCTCTTTGGGAGCCCAAGCTGCTCCCA  
GGCCCTCATTTGGGTATTAATTGGTTTTCTTTAGAGATTGCATGCTTA  
TCAATGGCCACTGGGCGGCTGGGCCTGGATGCGGTCCAGGCTTTGTATG  
Contig 65 (661 bp)

TCCCACGACCTGCCCCCTCCAGGGCCACATCTGGCGACACCGTCGCAAGAG  
TTGGACCGGCTGGTGTGGCCACAGCCTCAGGCCTTGTCTGGCCGCCAG  
GCCGGCTCCAGGCTCCAAGGAGCTCCTGCCTGCCCTCCGGAACCCAGCA  
CCCCGGGCCCGCTTCCCCACAGACCTGTTTTCCAGGTCAAGGTACAG  
CTAATTTGGGCTTAACTGGACAAGGAGGCCTTATCTGGAGCAGGCTCCC  
GGCCCTTTGGCCTCTGCCCTGGTGGGAGGCCTTCCAGAGGCTGTGTGT  
TGGCGCTGACCGTGCAGCCTGAGCTTGAACCCGGATAAGGAGGGACCCC  
ACCTGGGCTGGAGCCAGAGGCCCTCGTTCCCCAGCTCCGCAGGGTTCTC  
ACAGTCCCGCCCTGCCCTGGGGACCCTGGACGTCCCCAGCAGGTGAAAG  
GTCCAGATGCCCTCTGACTAGAGGCTCCTCCGCTGTACAGCATGCTCCCT  
TCCCGCACCGAGGACGAGACCTCAGCAGCCCTGCGTGGCCTGGGGTGCGG  
ACCCCAAGGCGTCTCTGAGTGTGTTCTAATGGGGAGCCGTGGGGCCTCAA  
CAGTGGGGGTGGCACTTGGAGGGGAGCCTCCCCACAGCTGCCCCAAGATG  
GGCCCTGGACT

Contig 66 (500 bp)

TTTGTGGATGAATGAAATCATGAGAAAGTGATTGGACCGCCCCGTTCGT  
CCAGCTGCTTGCCAGCTGCTTTGTAAAGATGACCTCTCACCTTCTCAGAG  
GCCTGGCCGGCCGAGGTGGCAGTCAGCTGAGATGCCATGCTTGTGTTGGC  
ACGTGGGAGGCCCTGTCCACGGCGTGGGTGCCTCTTGTGTCTAATCAGG  
GTCAGGGGGAGCAGCAGGTGCAGGGCACATGTGGGGCCGGGGCCGATGTC  
TGGGGAGGGCGGGAGGAGGGGTGTGCGGAGGCCGTTGTGGGGGTGCAGG  
GGACAGACCCAGCGAGACCCCTCCCTGGCCAGGCACCAGGACAGGTGATG  
GGGGGCCGCTCCGGGGCGTGTGACAGAAGCCTCTCAGAGGAGGCCCTCC  
CACGGTCTCTGACCATCAAGGGACCGGGGGCGTGGGCCTGGGGGTCAC  
ACCCAGCTGGCCGGCCAGCCCGGTGGGGTCGGAGGCCCGGGCAGTTTAC  
Contig 67 (550 bp)

GGGCAGGAGGGGCCCGGGGCTGGTGCAGAGGGTGGAGGTGGTGCAGGAGG  
GTGTGAGGCAGGGCTCACTGAGCGTGCGGGCTGGCTGTGCCCTAGAGTG  
GTTAGCAGGTGCCCCACCCTCCAGTGTGCTCTGTTACCTGTGCCTGG  
CTCAGGGTGTGAACTGAGACTCGGGTGTTCATGAGCTTCCAGGATG  
AGAATCAGCAGGCTTCCCAGGCAGGGCTGTGTCCGGGGCTCTGGGCTCTT  
ACCAAGGAGGGGACACCCAGGGACAGCCCTGCTTGGGGGTGTGGGGCTGG  
CCAGGCTGGGTGGTCTTCTGTGGCTGGCAGCCCTTGGCAGTACCCCC  
TTACCCTCAACTGCCCCCTCAGCTGAGACACGACCTCCCTGCAGAGCCCTG  
TCCACCCAGACTCACTCGCCTCCTCCAGGAAGCCTTCCAGGGCTGCCT  
CGCCCTGGTCTCAGCAGGAGACAGAGAGAGAGGGTGGGCCAGGAGCAGA  
GGCAGGCAGCCAGAGGGGAAGCCCAGGGGCCCTCACTACCCCTGGGGCC  
Contig 68 (500 bp)

TTTGCATTAGCTCGTACCCGGGATCCTTCCCGGGGCTCTGGGGGTGGG

FIGURE 6, CONTD.

GGAATGGGGGTGAGAGGCTGTCATCTGCCTGTCCTACCTGCTCTCAC  
AGGCTGGCCCTGGAGCCCTGGCCTCCTCTAGGGGCACATCAGGTTTTGG  
GGGAGGCCCAGCCACCGTCCCACCTCCAAGACCACAGCTGGGAGCCTGC  
CCCCAAGCCTAGACCTAGTGGGGCTCCTGCCAGCCAGGCCCCACCTTC  
ATGCTGCCACCCACCAAGGTGGGACAGTGCAGCCAGGACATCCAGCTTCT  
GGAGCTGCCCAGGCTCAGCACAGGCTGGTACCCTAGGGAGCAGGTCACC  
CAGGGCCGCTGGCGAGGCTGCGGGGACGGGGGTAGGGTGGGCAGCAA  
AAGAACCTCTGAGCTGGGCCGGGCGGGTGGGTGAGGGCCGGGGCCGCG  
GGCTGTGTGCGTGGCCCTGAGCCCGTGCAGACGCAGACCCTGGGTGGGT

Contig 69 (550 bp)

TGTGCTGCTGTGGCTGTGGTGTAGGCCGACAGTGCAGCTCTGATTCCGA  
CTCCTAGCCTGCGAACCTCCATATGCTGCTCTAAAAAGACAAACATAAAA  
TAAATGGGTGCGCTGTAAATTTGAACACTCTGCCTCCTCCAGAGACGAG  
GCCGAAACAGGCCCTCTCTGAAGGTCCACCTGGCAGGAGGAGGAGGCCA  
GCCCCGTGGGGGGCAGAGAGAAGCCCGATGTCCCAGACACACACGCACA  
GGGACCGTGGCCCCGGCTGCCAGCCCCGGGGGGAGGGCAAGGCCAGAG  
ACTCCACGACAGCCACAGGACCTTGGTGGCCACAGGACACAAACACAGGT  
GACGGTGGGTGAGGCTGGCCTTTCCCCCCTGGGCACGAGCACAGGACA  
CACAAGAGCCCCAGCGTGTGACCGCCACGCCAAGGAGCCTGGATGAAGC  
TGGACACCGAGAGTCCACACTGTGTGATTAGGCTGACGTGAAGTTTAAGA  
ACAAGCGGGTGGCTCAGCGCTTGAAGGCCAGAACAAGCCGGGAGGGCAG

Contig 70 (1300 bp)

ATGTCAGGATAGTAACCTGGGGTGTGAGTGCAGTGCAGATCCTTAA  
CCACTGTGCCACAAGGGAACCTCCTTGACCTAGAATCCTATACCCACTGCA  
AATATATTTCAAAAAGGTAAAGTCTGAGCAGAAAAGCAAAAATGGGAT  
AATTCATTTCTGGAAGACCTTCTTGTAAAGGAAGTTTTTTGGACGTGA  
TGAAGGTAGAACTCGGAGGCACACAAAGAAAGAAAGAAAGAGCAC  
TGGAAACGGGACCAATAAAGGTAAAAATAAAGTTTCTCTTTCTCATTT  
TTTAATTGCTCCAAAGATAGCTGACCTCTAAAGTAAAAATAGTGGAAA  
TGTAGCATATGTCTCTAGCGTAATTTAAAGTATACTTATAGCAATGATA  
GCCCAATAAAGGAGGAATTGAGAATATACAGTTGCTGTGTTCCCATTTGT  
GGCTCAGCAGTAATGAACCTGGCTAATATCCATGAGGATGCAGGTTCAAT  
CCCTGGCCCTCACTCAGTGGGTAAAGGATCCAGGTTGTCAGTGTGATGTG  
ACGTATGTACAGACGTGGCTCGGATCTGGCATTCTGTGACTGTGGCTG  
TGGTGTAGGCCAGCATCTGCACCTCCGATTTGACCCCTAGCCTGGGAACC  
ACCATATGCTGCTGGTGTGGCCCTAACAGACACAAAATAAAATAAAATA  
AAAGAGAGAGAGAATATACCATTTGTAATTTCTCACATGACACAAAGAG  
CAATGTGATATTATTTGGTATATGGTGATTGATTCAAGATGTATATCATA  
ATATTGATTCAAGATGTATATATTTCTTTTCTAAAAAAGAGATTATACA  
ATAAGGCAAGAGTGAAAATAAAGTGGAATGCTAAAGAATAGTTAATCCAA  
AAGAAGGCAGAAAATGGGGAAAAGACATATAACAGATGGAACAAAATAAAA  
AAGAGCTAATGAGATTGTAATAATTTAATCCAAACATACAGATAATCCCAT  
TAAATTTAAACACTCTCAACACATTGATTAAGAAATTTGTCAAATTGAA  
TAAACAAAGCAAGACCCCACTAGATGCAGACTATGAAAAACCACTTCAT  
ATAAGACATGGGTAGGTTTAGAGCAGAAATGATGGGGAAACCATGTCACG  
CAAACATTTGTCAAATAAAGCTGGTGTGGCTGTATTCTCTCAGACACA  
GCAGACTTCAGAACAAAGAACTGCAAAGGATGAAAGAGATACTGCATA  
ATGATAAAGGGATCAATTTTCCAAGTGCAGGCTCCAAACAACAGAGGTTT

Contig 71 (500 bp)

ATGACCTCATACTGAATCGAGCTCGGTATCAGGGGATCTCTCAGCTGGGG  
GGGAGGGCAATGGGGCATTGTCTGAGGATGCCCCAGGGCAGGCCCATTG  
GCTGTTTGGTGCCCATGCCCCCCCCACACCCCGGAGTGGCCCTGCTG  
AGCCTGGGACCCCTCTGGGAGTTAGGGATTGGGGGTGGGAACAGGCTT  
TGCAGTAATTCCAGCCCCAGGGCCCTTCCCTCCCCGCCCTCAGGACCCC  
CAGCCCCGCCCCACACAGTCTCCACTGTGACAGCCTCACCCTTGGGTCA  
AGTCTGTCTCTCCGGCCCCGCTGGGCAGTGGAGCCAGCTAGGTGAGA  
GGCAGAGGCCACTAGGGCGGTGGGCAGTGTGAGGACAGAGGGGCTGGG  
TGGCCTTGGACGAGGCCAGCGACGCTGAGACAGTGAAGAGACTGACATA  
CTTTCCAGGGAGGTTCCCTGAATGTCCACTTCTTGTGACATCGGGTGAC

Contig 72 (550 bp)

AAGTCCATTAGGGAAGGGATTTGTGCAAAACACAGAGACAGGTGCAGGGCT  
GGGCCAGCTGCTGGGCTGGGGCTCCTCAAGGCGCCCGTAAACCCCTCCC  
TGCCAGCCGCTGCCGCCAAGGTCTGCTGTCCACCCCGCCGGGCTGCTG  
TGTTCGCCGCGTGTCTCTGCGAACCCGACTCCCGTTACCCCTGAGCAC

FIGURE 6, CONTD.

TGCCTGGAGGCCGGCTGCCAGGCCGGACGGGGCCCTCAGGGCTGGGCTGG  
CTCTTGGCCTGTGTTTCATTTCTGAGCAGGTCTTCTCAGTGGGGGGGGC  
CTTGGGTGAAGCAGGCATGTGCACCACTGGGGCCCTGTCCCCAGTGGGCA  
TCCTGGGCGCTTGTCTGGCCCCAAACCCCCAGGCCGTGTGCATCATACC  
TTCACCTGAGCCCCAGCCGAACCCCGACATGTGCTGGGGGACCCTGGG  
CACAGGGGTGAGGGAGCAGTGGCCTTGGTGAAGCCAGCCTTGGCACCT  
GGGGAGGGGTGCATCTGGCATGCTCTGCTGTAACCAAGCCAGGGCAGG

Contig 73 (950 bp)

GACGTGCAGTAGCCATGACCTCTACGGCCCCCACTGACCAGCCCGTGTCC  
TTGTCCCAGAGACCGACCCCTAAGCAATAGGATGCAGCAGAAGTGACAGAA  
CGGCCTCCGCGATGAGGTGCGCAGAGGGCTCTGGCTCTGACTCAGGCCCCCT  
CATCCCTCGCTCTCTTGGAGCAGGGCCAGGTAGGGGCCCCCAGAGACGC  
CCTAGAGGAGGTGACGGGCAGCCAGCCGCCCCAGGAAGGCCTGGGGAC  
ACCAGGGAACAGAACGGCACAGGCTCCTGGCACAGTCTCCCAGGAGCCCC  
CTGGTGGCAGAGAAATCCTGACCGGCCAGTGGAGGGGGCTGGGGCGGGG  
CTCGGGGAGGAGGGACTGGGTGAGGCCGTCTGACTCCTGGCTGAGCGCCG  
CATACTTGTCTGCCCTGCCACGATGCCGGGCCAGGCCTTCCGCACGGACCC  
AGGCTCACATTGCCCCCTACATGCCACTGTGTGGGAGTTTGGGATGGTGTG  
CCCGCTGGGGCCCGGGGTGAGGGCACGCTTCCCAGAGGAGCGGGTTCCAG  
AAGGCCCAGGTGGAGAGGCGATAGGAGGGCTCCAGGGGGCTTCCCAGGCC  
ACCTGCGAGGACCCCTCCTGGGGGAAGGGAGCGGAGGGAGACAGCCGGGT  
CCCTTAGGCCAAGGCTGAGTTGTGACCGCAGGGAGAGAGAGAAGGAGCA  
CCCACAGCAGGGCAGGGGCTGCGGGAGGCTGTGCTGGGTGGCCGGGTGGT  
GGGTCTGGGGGCCAGGACCGTGGGAGGCTCGAGGGGGAGCAGGCACGG  
GAGGGGCCCCCTGGACGGCAGAGTCCCTGCTCCAGCTGCCGCCCCGACCCC  
AGGTCCACCTTCATTTACAGCCTGGCCCCCGGCCGCTCTGACCGGCCCT  
GCCCATGCAGGTGTAGCGGGGCAGTGAAGGCCAGGCTCCGGCCGTCCCAA

Contig 74 (450 bp)

GCAGGCCTGGCAGCAGGGAAATGATCCAGAAAGTGCCACCTCAGCCCCCA  
GCCATCTGCCACCCACCTGGAGGCCCTCAGGGGCCGGGCGCCGGGGGCA  
GGCGCTATAAAGCCGGCCGGGCCAGCCGCCCCAGCCCTCTGGGACCAG  
CTGCGTTCCCAGGCCGCCGGCAAGCAGGTCTGTCCCCCTGGGCTCCCGTC  
AGCTGGGTCTGGGCTGCTCTGCTGGGGCCAGGGCATCTCGGCAGGAGGAC  
GTGGGCTCCTCTCTCGGAGCCCTTGGGGGGTGAGGCTGGTGGGGGCTGCA  
GGTGCCCCCTGGGCTGGCCTCAACGCCGCCCGGTCCCGCAGGTCTCACCC  
CCCGCCATGGGCCCTGTGGACGCGCCTCTGCCCCAGGCTGGGCCCTTGC  
TGGCCCCCTCTGGAGCACCCCGCCCCCGGGGCCAAAGCCTTTCATGAACA

Contig 75 (1363 bp)

CCTCCAGCTGGGCCCGGCAGGGCACCGTGCCCTCAGGGGACACCACGGG  
GGGCCACAGTGGCCTCTCCTGCTCCAGGCTCTGCTCCCGCCTGGGGCCCC  
CTGGGCCGCCCGCCCATGGCCAGGGCAAACCTCCAGTGCGGCTGCCCGTC  
TGGGCAAAAGAGGCCGCCAGGCCCGCGTGGTCTTAGCAGGCACTGGCGGA  
TGCCGNTAACTAACCATTTCTTCCGCAGGAGTCCGAATCTGCTCTGACCA  
CGGGCCCTAAAAATCGCTCCTGGCCCGCAGAGGATCCCCGAACAGCGGGG  
CTGCCTCCTGCTCCTCCTGCTGCCGGCCGGCACTCGGCAGGCACGTGCCCTC  
GTCGTCCCCAGTCTGTCAACCGTCCCGTTCGTTACGATCCCCAGAGTCCCA  
CGCGCGGGCAGCTCTTTCCACACCCCGCACGGCCCCGGAGCTGCCTGGGC  
ACCCAGATCGCCCCCTGACGCCTTTGCTCCTAATTCTGCTGAAATACACAT  
AACGTCCTCCTTGAACGTTTGTCCATTTTACGGGGACAATTCTGTGGCCG  
TAGGTACACTCCCCCTTGGGGCGCAGCCATCGCACCATCCGCTTCCAGGAG  
GTCCCGTCGTCCCAGATGGACACTGTCCCCACTGATCCCTAATTCCCTGT  
CCCCCCCAGCCCTGCCCTTCTGTCTGTGGCCCTGGCGCCTCCAGGGA  
GCCCTGTGCGTGGGATCACAAAACGTGTGTCCCTTTGCGTCCGGTGTGT  
GTCTCTGAGCATCCGGAGCTTGGGGTGCTTCCACGCTGCGCCTGTGTGAG  
GACGTCCCTTCCCTTTTGGCGGTGCGCGATGCTCCCCGTGGGGCTGCCCA  
CACTGCGCGTGTTCGCTCATCCATCCACTAAGGCTGAGTTACTTTTGGCG  
GTTGTGAATACTGCTGTGTGAACACGGGCGTGCAATAACCTGCTGGAGGC  
CATGCTCTTAGGCCTCTCGGGGGGCACACCCAGAGCGGATATGCTCAATA  
AGGTAATTCTGTGTTTAGCTTTTGGGGAACCATCAGGCTGGTCTCCAGA  
GTGACGGAGCATGCGTCGCATTACAGGAATGGTCTGAGGCTTTGAGG  
TCTCCACCACTCGCTTCCATTTTCTGTGCGTCACAGCCGTGGAACGGC  
TGGGTGGTGCCTCTGTGTGGCTTCAATGTGCTTTTCTTTTCTGGCTAT  
GAGTTGAGCGTTTTTTTATGTACTTGTGCTGGCCATTGCGAGGGTTTTTGGG  
GTTTCTTTTCTTTTTTGCCTTTGGGGACGGCGCCAGAGCGTATAGAACT

FIGURE 6, CONTD.

TCCCTGGCTGGGGACTGAATCAGAGCTGCAGCTGCCAGCCTAGCCCACAG  
CCGCAGCAACGCA

Contig 76 (500 bp)

TCATGCCATCGCCACCGCCCCACCCGACGTTTCAAACACCAGAACCA  
CCCCCTCGGGCGGCAGAGAGAGGACCGGAAGGAGAGACAGCCTGGTCCCAA  
GGCCTCGCCCCGTCTGTGTCTCCGAGCGACATTTCTTTCTGTTTCCCTC  
CTCCGCGGTCCAAGTTTACCCATCAGAGGCGCATTTGTTTTATCATCTG  
AAAAAAAATCTCTGTCTCTTAATAAAACACAAGAAAAAGTAGCCTTCGA  
AAGAAAGCACATGAATGATATGTCTGGCGACAGTGTGGCGGCCTCTGA  
GCCGTGGTGGGAGGTGGGAGCCAGCGGAGCCCTGACCGATCACGTGACC  
CACGTCTCTCCTGCACAGCTGGCTGCACCTGCACGCGGTGACACAGGGAC  
CCAGCCTCCTGCCAGCAGGTACCCCCACCCGTCCTCTCTGTGGAAGG  
GGCAGCGTTGCCTTCTGAGGGTGGGCTGCTCTGAGGGGCGTCTTTGGCC

Contig 77 (626 bp)

GCCATGGGCTGCGGCGGTTACGCGGCTTGCCGGCCTGCCTGGAAGTCCC  
ACAGGACCAAGGGGAGGGACGTCAGCACAGGGGCCCGGGCACGGACGG  
TGCCGCCAGCGCCCCGGCCCCGCCCTCCAGACAGGACGCCCCGTACC  
TTGCGGGGACAGCCAGCCTCGTGGCCTCGAGCAGAAGAAGTGAGAGTGGG  
GTGCACAGGGGCCCCCGGGGAAGGAGAGGGGACAGCGGGGTGAGCGGG  
TGCGGGCGTGTCTGGGACAGCCCTGGCCTCTTGGCGCCTCCCTCCCCG  
TCCTTAAACCGGGCCAGCCTCTGGGCGCTCGACCAAGGCTGTTTGGA  
AATAGGTGGACCGTGGCCCTGACCCGAAGGCCAGCGGGGACCCGAGTGGC  
GTCCCCAATGGATCAGCAGGCGCTGGGCAGCCTGCGGCCCGGGACCCG  
GAGACACAGGTGGGAATGGGAGGAGGAGGAGGAAGACGGGAGGAGAGGAG  
TGAGGACCAGCAGAAACCACGCCCTCTCTCTTCCCGTCTCGCCCTCGC  
CTCCGACAGCTCCGACTCGGCTGCAAGGAAAAGCCCCAGCCAGCCCGC  
CGCCACCGGGGGGGGGGGGGGGGGGGGG

Contig 78 (500 bp)

TACTCGGTTTGTGTACCACTGAGCCACAAAGGGAGCTCCTAAAAATAATA  
ATTTTCTTAAAGCCAATGACATGGAGAGCAGTTAGGGTGGAGGCTGGTGG  
GTGGTGGGGCGCGGCAGGCGCCTGAAGGTCTGAGTGGCACCTTGGC  
CGGGGGAGGTGGGTGGGCGAGGGGTGTTGAGAAGGGGACGGGCCCTGTGG  
GGGCAGGAAGGAAGAGCCAGTGGCTCCCAGTCCCCTGACCTTGCTGCCTT  
GAGCCTGGTTCTCCCCAAAATTCTGTCTGTGTCCCTTCACTTACGGAAG  
CTTGGGGCCCGTTGCCAGGGAGACAGATGGGCTGGTGACACCCAAAATGA  
GCCACCAGGAGGGGGGCACTGACTTTAGCCAGCCGGTCACATCAAGAAGC  
AAACAGGGCCCCCGCTGCTGTAAAGGCAGCTTGGGGCTGGGGTCCGGGAG  
CACCCTTGGGCTGGGGAAGGGGGTCTCTCAGGCCCCCGGGGAGGATG

Contig 79 (427 bp)

TCTATTCCCGTGGCCGGAAGAGGCTAACCGTACATTGACCGGGCATCTG  
GCGATGTATCACTTCTCTCCAACCGAACTTCCCGGCAAACTTGCTGCG  
TGAAAACGTTGCGGATAGCCGAATCTTCATTACCGGTAATACAGTCATTG  
ATGCACTGTTATGGGTGCGTGACCAGGTGATGAGCAGCGACAAGCTGCGT  
TCAGAACTGGCGGCAAAATTACCCGTTTATCGACCCCGATAAAAAGATGAT  
TCTGGTGACCGGTACAGGCTGAGAGTTTCGGTCTGGCTTTGAAGAAA  
TCTGCCACGCGTGGCAGACATCGCCACCACGACAGGACATCCAGATT  
GTCTATCCGGTGCATCTCAACCCGAACGTCAGAGAACCGGTCAATCGCAT  
TCTGGGGCATGTGAAAATGTCATTCT

Contig 80 (650 bp)

GGCGTTGCCGTGAGCTGTGGTGGGCTCACAGATGGGGCTCAGATCCCCG  
GTGGCTGTGGCTCTGGCCTAGGCCGGTGGCTGCAGCTCCGATTGACCCC  
TGGCCTGGGAGCCTCCATATGCTGCGGGAGCAGCCCTAAAAAAAAAAAAA  
AAAAAAGGAAGAAAAGAGAAGAAAAGAAAAGACAAAAGTCAAAG  
GAGCTCCCTGAGCGATGTCTGTCTACGAGCAGGTCCCTGGGAGCCTGAG  
GCAGGGTGAGCCTGGACCCCTGAGGGCCACTCCAGACTCAGTGCTCTCAC  
TGGCCAAGGTCTTTGGGGACCGGCTGGGGGCGCGCAGGCTAAGGAGGA  
GGTCAGAGGAGGGGCTTCAGGCTGCAGGGCCAGCGGACGCTCTGGGCCCG  
GGGCGGGGGGAGATGGCCTGAGGGCCTTGCAGGGGCTGGAGGGTGGGGG  
GCTTCTGAGAGTGGGAAGACGGGAAGCCAGGTGAGAGGAGAGGAGCGAGG  
GCTGAAGCTCCTGGAAGGCGCTGGCTACCCCCAGCTGGCCCCCCCCGCTG  
CCACATTCAACAGCCACCCGGCCTGTGGTCTTGGCAGGGTCTTGGCAGAA  
AAGCCCCAAGGGCCCCAGCCTGGCCCTTGGGCCTAAGAGCCAAGCCCC

Contig 81 (550 bp)

TTAACCACGGAGCAAGGCTGGGGATCGAACCTGTAACCTCGTGGCTCCT

FIGURE 6, CONTD.

CGTTCGGATTTCGTTAACCACCTGCGCCACGACGGGGACCCCCAGGGCTGGC  
GTTTCCCTCTGTGTGCACACAGTGGACCTGAGCCAACCAGCAGGGCCTTC  
ACCACCACGGCGCAAGAGTCCGCGAGCAAGAGAGCAGTGTCTCATGGCTCA  
CTTTCTCCCCCTTCCCCGGAGTGGTGACAAAACCCGCGGCCACCGGACT  
CGGTTAGACAAGGCGGTGCCAGTGCCCCCGTCTGTACCCGACGCGCAC  
GGCGCTCTCCTTTCTTCTCGGGGCTCCACCACGTGTCTCAGTTTCCGC  
ATGAGAGTACCGCGGTGGCGGGGTGGTGGCTCTGGGGTCGGGGGCCGTG  
AGGGCAGGGCTGGGCTGGGGGAGGCAGGTCTTGCCCCATTACGCGGGGG  
CAGACTCCACATCACACGCTCTCTGTGCCTCTTGGCTGCCTGACACCATG  
GACTTCAAACAGGAACAGCCGTGGAGGCATTGCAGCCCAGGGCCCCGGTT

Contig 82 (550 bp)

TGACACCTCCAGGCAGGAGGTGCAGGCTGGGGTCCCAGGTAATGGTGTG  
CTGGCCTGTGGGGCGTGGGCTCAGCTCTTAGGATGGTGGGCTGGGCGCCG  
ACCCAGCAAGGACAGGGTGATGGCAGGTCGTGGGCTCAGCAAATGAGTGC  
CCAGGTTGTGGGGGTGGGCACTTGGGGCTCAGGGGAAGCTCATCAGCTTG  
GAGAGGGACGGGGAGGGAGGGGCTTGGCCAGCTGGCCAGATGCCTG  
GATGTGAGCACTCACGTGCCCCGGGGTCCACCTCCCCTCCAGTGCCATCT  
GGGCAGGAGGCTCCGATGCCTGTCCCTGGGACCGCTGTCTGAAATGAG  
GTTCACTTGGTGCCTTCCCCAGAGATGCTCGGTCCGGAAGCTGACGAGGC  
AGGAGTGCACAAGGGTCTGGGGAAATGGAGCAGAGTGGGCTGGGGCACA  
GAGGCTGCCCCAGCCTGGGAAGATGGGGAGCTTTCAGGGGTACCCCGC  
CAGCTTGTGGGGCCCTGGATACCCAAGGTGTGAAGAGGCTGAAGAGCGA

Contig 83 (984 bp)

CTGAGCCACGCTATGTAGATTAGACCCCGTCCGTCCCAAATTCTTCTCA  
AAGCTGTCCCGAGATGAGAGATGAGGTTTTCTGTCTCTCTCTCTCTCG  
CTTCCCTGGGATGTGCCCTAGGGTGGGAGAGGGTGTGTCCAGGGCTCA  
GCAGGCGGTCCCATCTTCCCAGACGGGAGAGATCCCTCCTTCTCGGCG  
CCTGTCCCCACGGCCCCACAGACACCCCCCCCCCGGCATGGCACCCAT  
GCACCTGCCATCGTGCCCACTAGGGGATGGGTTTGGCGAGACTGGAGATG  
GCTGTAGCCAGTGAGACATGCCCTGCCACGTAGCCTGACCCCTGGGTGT  
GCTCTGTGAGATCTGGGGACCCCCAGCACACCTAGGGATCATCTTTGCCA  
GCCTCCTGGGGAGCCTCTCAGAAATGGGGGCCCCAGAAAGGCTGGCAAAG  
GTGATGGGGAGCGTGGGAAGTCTGGCGGTTGGCGGGGTGGGTGGGGGCA  
GTGCGGGTGGGTGGGGGTGCTCCGGGTCGGAAGTGGTCCAGCAAGGT  
TTTGGACACAAAGTCAGGAGGAAGGAGTGACGAGGAGACTTGCAGAATTA  
CAGGTAGAATCAGGAACCCACATCGACGCCAATTGATCTATCCCCCCTT  
TGATTGTTTTCTCTCTGGGGCTTTTTTCCNTTTTTTTTTTTTTTTTTT  
TTAATCCCTCCTTAGCTTTTACGCGCTCAACACCAAATTAACGTAATC  
CCCACCCACGTAACAGGGGGGCGGTGACCCGAAGGACGAGGAGCACACG  
AAGCCACGGGACCGTCACTTGGCGGCACACGCGCTGTCTGCCCTCCGC  
CCATTTATCGCCCTTGAATTGATTTTTGTTTTGTCTGTCCCTGTGCTT  
GGGTAGAGTGGAAAAGGGAACCTCTGTGGGGGTGCCAGCCACTGGGCCCC  
CCAAAGATTTAGGGGAATGAAACGGCTGCCGCC

Contig 84 (550 bp)

TGCCCTGACAACCTGCCCTGTAGCCACACTCGCGACTAATAAGGCGA  
GAGGTACGCGGCAGCCCCACGGGGAGAAAGTGCTCCGTGCCCCCCACC  
CCTGGCTCTGATGGCCAGCCTGGCACCCCAAGGTGGCCTCGGCCTTCCT  
ACCTCCAAGGTCCAGGCGCATGTCCAAGCACCAGCAGAAGCTTCTCCAGG  
GTTGGTGCCTGCTCAGGGCAGAAAGCAGGGGTGAGGCTCCCAAAGGGCC  
ACTGGCACCAATGCCCCAGGCAGCCCCAGCGAAGGGGACAGCCACCCC  
CAGCCCGGGGACGCAGGCTGAGGGGACATGGGGAACCCAGAGCAGGGCC  
AAGGGGAGCAGAGCCCTCCTCCGGGACTTGAAATCTTCCGGGGGGCC  
CAGGGAGCTGGGGTCTGCAGAGGGCACTTTCAAATACGGCCCCACCCCA  
AATTGCCACGTGGGCCACAGGAGCAAGGAGTCGCTGCCAAAGTGGCCTGGC  
TTCAGCGCAGGAAGTCCCCCTCCTGGGGCTCCCTCCTATAGGCACAGG

Contig 85 (500 bp)

TGAGCCAGGGCCTGGCCAGCTAAGCCCTGGAGCCCTCCCGCCTGTTT  
CCTGCCCTCCATGCTGGCGGAGCTCGGCTTACTGAGCGGGGGCCAGGCCA  
GTGTGCGTGTGGAGGTAGATTCCACTCAGCTGGAGGTTGAGGTGGGCAGG  
GGGCCGAGACCCCTCAGGCCAGCTCTGGCCGGCCAGGTCCCTGAAGCTCC  
CCCGGCTGGCCTCCCGTCCCTGCCTCTGGCCTTGTCTGGCCCTTGCTT  
GACAAAGCTCTGTGGCTCTGCCTGCAGGAGAGACACTGGCTCCCCGCTC  
TCGGATGAGGACGGGGCTTTTCTGCACAAGTCTGCCCCAGAATGTTTGG  
GGCGCCAGCAGCTGAGCCAGCACGTCTCCCTGCCCCCTGGCTGGACAC

FIGURE 6, CONTD.

GAATCCCGGCATCGAGGCGGGAAGGGGGATGGAGGGATGGGGCCTACCCA  
CCCCTGCTCCCCACCCAGAATAGCTGGGCGGCCCCATGGGAGGCCGCC  
Contig 86 (913 bp)  
CTGTTTTACGTCTTCTGAGGACACACCCAGAAGAGGGGCTGCAGGCGCC  
CATGGTGACTCCATGTGTCTCACTGCTGAGGCCTCTGCAGACCGTCTCCCG  
CAGCAGCCGCACCCGTTTCCATGCCACCAACAGCGTGCGAGGCCGCACTG  
TCCCCACGCTGTGCAACTGTTTTGAATCTGAGTTATATAAGCAACAGAC  
GCTCCTTCAAACACACTCACGTGCACACGTGCGCACAGGCGCACAGACAC  
ACACACGGAGTAATAGGCCTCCCCCCCCCTCCCTGAGCCCAGAGGGGGCCT  
GGGGCCCTGGAGCCTGTGCTTTAGGGCCTTTAGGAAAGCTGGTGCCTCC  
CAGAGGGGCCGCCCGAGCGTTGGCTTCCCAAGTCCCCACCAACCCTCGA  
CAGACTCAAACGTTGGTTCTTTCGTGCTTTTGCCCAAGGGATGGGCCCCG  
AGGTGGCCCTGCCTGAGGTTTCAGCCACGCGCCCAAGGCACCCTTTCTCT  
CCCGGTCCCCGGCCACTTCATGGGACAGCGGGCCTTCCCCACGTTGTCC  
CCTGGGTGTGCTGCTTTTCGTAATGAGACGGAGGCAGGTGCACCTGTCC  
TGGGGTGAATTCTCTTCTGCAGGAACGCTTCCCCGGCGCCTGGTCTGT  
CTGTTCTCGGTTGTTGGAACCTCTCGTCACCAGAAAGGGTGGCTCTGAC  
GTCGCCCTTTCCCTCCGTGGCTTTTGCAGTCTGGGTCTTGTGGGGAACC  
TGCCCCAAAGAGGGGAGTGACCCCCACGAGGGAGACGTAGCTCCTGTGG  
CGACAGCACCGGGGGCCCCCAGATTTCATGGGGTTACGCTCACAGTCGCA  
TGACGCTGCCTTTGGACGAGGGCAGCTCAAGGAAGCTTGTTCCTGCCA  
CGAGCCACAGGCA

Contig 87 (650 bp)

TCCACACCTGTGGAGCCGCTGCCTCGCTGATGCCCTCTGCCAGCTGATG  
GTCAGGTGCCAGACTTGGGGCTCAGTCCAACAGGGGGCCACAGGTGCT  
GCACCTGGGCAAGGGAGCCTGTGCGCAGGGCCTCAGGTGTCCAGGCTCG  
CTGGGACCGAAGCGCACTGGGTCTGGACTCCGGGCTTCCCCAGGGGCTG  
CTCGGGGGCCACCTGGAAATGAAGCCCCACCTGGCTCATAGGGTCCACGTG  
AGGGCCCTGAGGCCACCAAGCCACCAACAACACTCAGTTAAGGGAGGGGAG  
CTTGGGGCTGTGAAGCTCCAAGCGGGAAGCGGCCCACTCAGCACTGCCT  
CTCTGCCAGCCAGCCGCCAGCTTGCTGACGTCCCAACCAGGCCAGGGAC  
CCTGTCCACAGATGCTGGGCCCTTCCAGTCTCTGCTCCCTGGAGGCGCT  
GGGCACTGTGTGGGCACACAGCCCGCACCCGCTGTAAGGAAGGGAAAGG  
CCCCATCTCAAAAAAGCCGTGGGCAGGTGGGCCATGATGGTCTCCGAG  
GCAGGTCTCTCTGGGACCCCTTGCTCCCTCGGGCTCGCCCAGGAGCCGCC  
AGGTCTGCCCTGGATTAACCTTGCCCCGCATGTCATTTCAAACCTGGCTT

Contig 88 (700 bp)

TGGGGCCCTTTGGGGCCGGAGCGGCCAGTCTGCTGGGCCCGGAGCAGGG  
GGTCTCTGTCCGCAGGGAGGGGGCCTGGTCTCAGGGGAGGAGAGGAGCA  
GGTCTCACCTGAAAGGATCTGCCTTCTCCTCAGGCCTCTGGGATGCCTGG  
GCAGAGAAACCAAGGAAGGAAAGGCCCAACTTGCTGGCTGGTGGGGATGGGG  
CCGGGGGTGCTCCCGGCACACCCCCCAACCCCACTTAGTGGCCAA  
AGTGGGTGTGATGATGGCCACTGACCTCACGGGGGCGCAGGAGACAACAA  
AATTCAGCCACTCTTGGGGGAAGGACACTTGTGGCCTGAGTCTTAGGGG  
CTGAGTTTCGGGGGGGACCCCCAGCTCTCCCCCAAGTATGAGACACCCTG  
CCCACTCCTCCAGCTGCTCCCCAAACCCAGTGCTTCTGGACGGGCATCT  
CCCCGCTGCCCTGCGAGCCGCTGTCTCTGACCATGTCCCTCCCCACCT  
CCCCCTCTGCAGGGCCAGGCCTCCAGGGAGCAGAGCCGAGGCCCAACCTA  
GACTGAGCTGGGGACCGAGACCCCAAGTCGCCACCCGGTCTCTGCGTTAG  
AGAGGGGGTTCCGGGGGGCACCTGGGGCGGCACTGGGGGGCGGGAAGGA  
GAGCCCTGGGCCGTTCTGGGAAAGGTCTGGGAGGGAGGGAGGGGTTTTGC

Contig 89 (1400 bp)

GCACACCCGGAGAACAGAGGGAGGGGTCCTTACCAGTCTCAGGGTTTTTT  
TGGGGATTTCTTTGAACCTTGCCCTATTGGTTTCGAGGCTTCTGTTCTCTC  
CAATCCCCCTTCTGAACCCCCCAAAATGGGTTACGCCCCACCCAG  
CCAGAGGAAACCAATTGGGGGATTGGGGGGAGGCGGGGCCAGCAAAAGCC  
TTGGGCCCCCAAGCCCCCTGGCTTTGGCCTCTGGCCTGCCAGGTAGGGGG  
AGGGACGCGGTGACCTCCGGGGGCTGGCCACGACTCTGCCCCACCCC  
CAGGGCAGACGTGCACAGGAGGGGAGAGGCTCCGAGGAATGAGGCCATCA  
AAGGGACAGGTGAGGCCACGAGCCGTGGGACCTGGAAGTGTTTAGGGCCT  
GGGGGACGAGGTGCGGCCTGCGGGCTCCGTGGTCAGGAGGCCCTCTGCC  
CACTGAGCAGCTCCCACTGGCACACGAGCCTCTCTGGGGTCCGGCTG



FIGURE 6, CONTD.

GTCTCCGGCAGGGGTGGGCTCTGAACGTCCAGCTCCGCAGACAAATCAGA  
 TTCCCCGAGCCCTGAGAAAGCCCCCTCCCCAGCCCGTCTCCCCACCTG  
 TCGGTGGACAGAGTGACCCCTGCTGACCCCTGCCCGGGCTCCCGCAGGA  
 GATGTGAGAGAGTAAGAGGCGGTACAGGACGGCCGGGGCGGCCCGGGCGA  
 GGTGCAGGTGTGTGGGTGTGAGGCTGGGCACAGGCTGGCACAGCCTCCCT  
 GGCCCCAGTCCCTTGGGCACCTCTGGGCACCTCGGTGTGCCTGCCTCCTGA  
 AGGGATCCACCCCTCCAGCCACCTCCTCTCGGGCCAGCCCCACCCACCC  
 CCGAGCTACAGATGCCTGCGCATTGCCCCAAGTGTCTGGACCCCTGGAG  
 CCAGGCAGCCACCCGCTCAGCCTGGCCAGACCCAGCGTTGCCCTTCACG  
 CCCTCCTCCTCCCGCCGGTCTCGCGCTCGTCTCCTCAGGTTGGAAGC  
 CCCTTCCCACCTGCCATCTTGCTGCGCCAGGATACACGGCTCAACTCA  
 AGGCCTCACTCCTCGCCCTCTCCAAGGCTCTGTCCAGGCCCCCTCTCTGAC  
 CTGGCACCACTGCCGCTCTGGCAGCCCCAGCAAACCCCTGCCACAG  
 TCCACGACAGTCTCTTCTGGCTCTGCCCCCAGGATGCTTCTAGAAGTGG  
 GGGGGGGTCTTCCAGCCACGCAGCATCCACTGGGCCCTGGGCTCCCT  
 CCCCAGGTGCCCCCTCAGAGCTTGACGCTGGTGACAGCGCTCTGCTCCGA  
 ACCCATGCTCCCTGCGCCCTTGGACCTGGTGAGATGTTGCAGGTCATTTG  
 GCTGCACCCAAAAGAGTGGCCCCCTCAGGGTCCCCCTGCGCCCCCTCCATC

## Contig 90 (350 bp)

GTACTGTAGGGCCCTCATTCGAATAGCCTACTAGGTCACAGCTGATCCACA  
 CCTTAGGCCATCACAACTTCCCAGAGGTAGTGCCGCTCCTGTCTGTTGAAC  
 AAGACGGTAGTGACTGCTGTGAGAGCTCAGATCTGGTGGGTCACTGACCG  
 AGTGTGGAACCTGGGGGAAGGCTGTGGGTGTCCCCGGCTGGGTGGCCA  
 TGTCATGTGCCCCCTTCTATCCCTTGGACGAGGCTGGTTCACTCGGCTCT  
 AGAGCCCCAAGCCCCAGCTGCTCTGCCAACCCCCAAGCCTGAGCCTCAT  
 CAGACCCACCAACCCATCGCCATGGCTACGCAGGACACACCGCTCTCCAC  
 CCCCACAGCCGCCCCACCTCCCCGAGGTTCCAAAGCTTGA

## Contig 91 (1464 bp)

TCCAGGACCTGATGCAGCAGCCACGTCCCGAGGCCCCCTCCACGAGGCCC  
 CTGTGTGACCAGCGCTAGGGAAGGGGACCAGGGAGATGCTGAGAACGGGG  
 CCTTCCGAGGGGGCAGGTGGGACTGACTGTGACCCAACTCCCCACCCC  
 CCTCTCCCGCTCCAGAGGGTGCCAGCCTGGAAGCTGGCAAAGTCCAATCC  
 ACAGGTGGGCTCACGTGGGGAGGCTGGTGGCCCCACCTGGTGGGGCCCC  
 AAGCTGCCTCTGGGCGGGGTGGGGGCTGCTCCCAGCAGGGTCCCATCCAG  
 CTTCTCCCTGGGGAGACTCACAGTTCTGGGAGAAGGGTCTGACTGCACC  
 GCAGCGCCCCCCCCCTCCCCAGACTACCCAAAGTTCTCTCTGTCATCGG  
 TGACTGGTCTCCGCATTTGCCAGGCTGGGCATCTGCCAGAGGATACGT  
 CCAAAGGCAGGGCAAAGCGGGCCCGTCCCCCGAGGCTCCCCACAGGCGC  
 TGAGGGCTGGGCTGGATCTCGGGGGGTGGAGGGGAGGACTCAGAAGGTG  
 CAGCGGGGTGGAGCGAGGCTGAGCCAAGGTGCACGCGAGGGCCAGAGAAG  
 GCCGAGGCGGGCAGGAGGAGAGCGCCAGCCTGGAGGGGGGTGGGTGCC  
 CTGGGCAGGTCTGGGGCTCAAGAAGAAGAGAGTGTGTGTCAGGGGGCTG  
 TCCAAGCTGCCCGGAGGCTGCCTGCCACCTCCAGGGAGCAAAGCAGGG  
 AGGCTGCAGCTGGCCCGGCCGCGCTCTCCAGGACCACGCTGGCCCAG  
 GCCTCAACGCTCCTCCCACAGCCAGGAGACCCAGGGCACCGGGTCCATT  
 TACCGCGGGCTCCGGGTCCGTTTGCTGCGCCCTGGGATGGACTGTGGGG  
 GCGGGGCGCTGTCTGGGGAGGAGGAGGTGTCTGAGGCTGGACACCTTGA  
 AGGCAGGTGAGAGTGACAGGTCCGTGCGCAGGAGCCTTCGGCTCTGGATT  
 CTGGCCCTGAGCGAGGGGCTGGCTGGAAGTGGGCCGGGGCTGCCGCAGG  
 AGAGTGTGAGGGAGAGGAGACGGGGTTGGCCCCGAGGTGCCGGGGTG  
 GTGCCCTGGAGTGCGGCTGAGCGGGAAGTGGGTGTGGCGTCTGGAGACG  
 GGGGGTCTGGGCTTGGGATGGTGACAAGACCCCCAGGTGGAGGCGGCC  
 GCAGAGGAGGCAGAGAAGCCAGGCCAGCCCCAGCCCCAGGCGGGAGGCTGGG  
 AGTCAGGAGGGACAGCAGAGCCCTGGGCTCAGTGTACCCGTTCTGGCA  
 CCTCGCCAGCGATGTCTGGCCGTGCAGTGGTGTCTCCCTCACCTGAG  
 CCCTGAGAACCATGCAGGATGCTGGTGTACAGCAGGAGAGGGCCAGGGC  
 CTGGGGAGGAGTCTTACTGGAAGGCCTTCTCCTTCCGTTTGACGAGGCG  
 GGAATGACTGGGGG

## Contig 92 (694 bp)

TGGAGCCAGGGCACGGCAGAGCGGTCCCGAGGCCGTGCGTGCTGACCCGG  
 GGGATGGGCGACCTGGGGGTGGGCTGTGAGCCAGGCATAGGGACCCCG

FIGURE 6, CONTD.

ACTTGGGCACGGCCAGGTGGGGCCGGGCAAGGGGAACAAGGACGCTGGC  
CTCCAAGGGCCCCACGTGGGCACAGAGGAAGAGCCGACCCAGGTTGTGGG  
CGCATGGAACCCCCCTCTGGGGGCCAGGAGGCCGAACGTCCAAGGGC  
TGAGGCTGGGAGGGAAGAGTCCCTTTGGGGGTGAGTCAGTGTCCCTTGTG  
GGTGGCCCCCTGCCACTGGCGGCACCTCTGACCCCAACTCCTTGCGGGTG  
GACGGTGGATGGATTTCCTGCAGCCTTCTTCTGGAATAGTCTCTGCCAT  
CCTCGGGGAAGCAGTGATTGCTCTGCCCAAGTCCAGGCCCCGCCCTGCAA  
GGTGCCCTCCACCCCAATGAGCCCCGGACAGTTCGAGGGCTTCTCACGC  
TACTGAGGGGTATGAACAGCTGTCCCCCTCGGAAAGTGGGGGACAGGCC  
CTGCCACTCCATCCTCGGGACGCCCCGTCTAGTCAGCACTTGTCTCCCTG  
CCTTGTCCCCCTGACCTTTTTTGGAGACCATCAAAACCTCAGCCTCTG  
CCCCAGGAGGTCAAGCCCCCGTCCCCAGCCCCAGACCAGCA

Contig 93 (900 bp)

CCAGCCCCATCCCCGGCTGGTCCCCACCACACAGAGCCCCGTTTCCC  
AGGGGACAGCACAGCCTGCCCCAGGTCTTACATAAAGTCACCTTCTCAG  
AGCTCCTGTGCGGGCTCAGGGGAATGAATCTGACCAGCATCCATGAGGAC  
ACAGGTTTGATCCAGGCCCCGCTCAGCAGGTTAAGGATCTGGCGTTGCC  
GTGAGCTGTGGTGGAGGTGCAAGACGTGGCTCAGATCTGGTGTGGCTGT  
GACTGAGGTGGCGGCCAGCAGCTGCAGCTCTGATTGGACCCCTAGCCTGG  
GAACCTCCATATGCCGCGGGTGCAGCCCTGAAAGGACAAAAATAAATAAA  
TAAATAAAGAAGTAAACACACCTTCTCTAGCCATAACCACCTGCCTAGG  
GGCGGAGGGCCAGGAAGCGGCACCCCCGCCCCAGGCTGCCCGTGGCGCC  
CGGGCAGGCGGGCTCAGCCTGCTTTTGTCTGTGATGTGAGCGGCCCCAGC  
CCCACATGGAGGGGCTGGGCTGCGCAGTAAGTCTTAACTGACGGGAGC  
TTCGACCAGCAATTACACAGCGGCATGCAGCCGGGAAGGGAAGTTATTC  
GTGTGTAGCTATTAGGCGCCGAGTGAGGGTGTGCCCTCGCCCTGGGCCCCA  
CCCCTGGGGGGAGGCATCACAGGGCTTTTGAACACCTGCCCATGAACACG  
GGGCAAAAGCCAGCCAAGGGGGCAGGTGCCTGAGGCTGGGAACCAACCCG  
TGTCTCTGAAATCCGGGGAATGCCCACTGCAGGCATGTTCAAAGGGTCAA  
GACCGGGGCTCTGCCTGAGAAGGACTGGCGAAGGCCAACTACAAAAGCGC  
ACCCCTCTGTGCAACCCCCCAACCAATGGAACAAACTCCAGAGGGGCCA

Contig 94 (550 bp)

AGTCTGGGCTGTGTCCATGGGGTTGCCAAGGTGCCAGGCAGAGACCTTGG  
GGACAAAGGTCTGTGAGCAGAAGGACATGGCCACGTCCCCTGCTCAGCA  
GGTGCCCAAGGCTGGGGTCTGATGCCCTCGCTGGGGTGGGGCGGGTTGAG  
GGGCCAGGCCAGACACCCCTTCGTCCCTGCCGAGTTGTTGCCCTTCTG  
TTCCTGGAAGCCCCCTGCAGGTACAGGAGGCCCTGGGGCTGACGCTG  
CACCTTCTGACACCTGTGGTCTTGGGGATGGGACAGGACAGGGAGACCC  
GGGGCTGGACGGAGCGGGTAAGACAGAGAGTTGACTCTGTCTCGAGTCT  
GTGCAGGGCTGTCCCGGGCTTGGGCTTCGTCTGCAGGGCCTTTCGGGTCA  
GGGTGGCCTCAAGGTGACGAAGACCTGGTCTCGGGAGTCTGCAGGCGCA  
AAAGTTGGAGCCACCCCCCGGGGAGGGGGCCCAAGGACAGGAGGGCC  
CAGGGAAGTCTGGGGCTGCAAGGCCGTCCGGGCTGGGGAAGGCCAAGGT

Contig 95 (1200 bp)

GTTTGTCTCAGCAGGCAAGGGCCTCCGAGGCCTTAATAGCCCATATGA  
CAGCGCCCGCTCCTGGCATGGGGCCCCGCTGGCATGGGGCAGGGCAGGG  
CAGAGCAAGCAGCATGCAGCTTCTACCTTCTCTCTGACCTCGTGGCCCT  
TCCGAGGCCTCAGGGGGTCCCCGAGTGGGACCCAGCCCTGGCTCTCCT  
CTCCAGAGCCAGGCCCAAGGCTGGGAGTGGCCAGAGATGAGGGTGGCCG  
AGCAGGGCACTGCCTTGGCGTCCCATCCCTGGCGCCTCAGGGCCGTACT  
GTCCAAAACCAAAAGAAAGCAGTCAGCAAAACTTCTCCAGCAAGCTGGG  
GTCAAAGGTGCTTCCGAGGCGTGATCAGGGTGGCCTTTGCTACTGTAC  
CGTGTGCCCTGGGAGAGGCACAGGGACACAGACACACCTCCGAGAACC  
TGGGGCTTCCAGGGCGTCAGGCTGCCTGGGCCATCCCGGGCCCCCTGTGGT  
CCCAGGATCTCGCGGACCGTGAGGCCTGCGTCCCACCTCTGCCTGGGA  
CAGGCCCCACAGAGCTCACAGCCAGGGGACCGGGGACAGGGCCCCGCTG  
GGCCACCTGCCTCCAGCCTCACCCAGCCTGGGCCCCAGGCCTGTGCCTGC  
GACACCTGTAGTCTCAGGACGGGCGCGGACAAAGCCGCCCGGCCCTCC  
CCCGGCTGGGAGGAGACCCGCTGGCCCTGACGTGTGGGCTGTCTCAGAGC  
TGAAATGTACAGCAATTAGCCCTAACGAGGCCGAGGGAGGGAGCGGGCGG  
GGAGGCCCGGCGAGGGGATCCACAGCCGAGGGGCCGGAGCTGGCCACCC  
CACCGGTGCTATCCAGGCACTCAGGGATAATTGGGTGTTAGAAGTCAGG  
CGGCAGCAGAGAGCGGGCCAGGCGGGCTGTGCCCCCTCCACCGCCCC  
TTAACAGGTGCCCCAACACGCAGGTCTGGGGAGATGCTGAGGTGCGCAAG

FIGURE 6, CONTD.

[illegible]

Contig 97 (1350 bp)

CCCGCCTTATTTTTAAATTTCCGAAAACAAAAACCACACCTCTCCCGTCC  
CCGAAATTATTTTGGTATAGTCTTATTCAAAGAAGTCTGCCACTGAAGC  
CCACTTGTCTGTGCCGGGTGCTTTGGCCAAGGGCCCTGACGGGCCAG  
GCTGGCTCATTTCCGCATCCCGCAGAGGCCGCTTCATACCTCCATGCGG  
GAGCTCTGGCTTCCGGCACCCGGCTGTGCCCTCGCTGTGCCCATGGAATGC  
TTTCGCAGAAGCATAGGGGCCACAACATGGGACAGCCTCGCTCTGCTCGC  
TGTGGTTCCGCTGAACCTCTCAGCTGGACATCTGGGCAGCAAGCACCCCA  
GCTTTGCTTCAAGGCTCTGGTTCCAGGCTGGGCCCTCCTCGGCCCTGCCCG  
CTGGGTGCCAAGCAGGCGTGGTCCGGCTGTGCCCCGGGTCTATAGAAGC  
CTCGCAGGGCTTCTACAGCCAGGCTGGGATTCGGCGGCTGCCCGGAC  
TGAGGCCCCCTCTGAGTCTGACCCCCCATCCTTCCCTCCACACAGCCC  
CCCGCCCCGCTTCTGCTTCACTGAGGCCCCACCCTGCCTCACTCGCTGA  
CATTTCCAGAACAGGGGGTTCAGGAAGCCCTGAGCCTGCAGGGGACTCA  
GTGACCAGCCGCATCTGAATTTTCCCTCCTTCTGATCTCTGGAGACACGT  
CTGGCTCAGCCTGGCTCGAGTCCCTGAGCTGGGACCAGGACAGACCTG  
CAGATGGAGGTCTGAGCCTGGGCAGGGCAGGGCCCAAGGCTCAGGGAGAA  
ATTGCAGGTGTGAGATCAATGACCGGAGCCTGGATGGGGCCGCCCTGGCC  
AGGGCAGCTTTCTCCCTGCAGCTCCCTGCCACTGTCCCCCCCCAACTCTGG  
GCTCCTGCTCTGGACCCAGTTGTGTGTTCCCCCTCCTCCAGCCGAGCCAC  
CCTCCCCCATCTGCCCCCCCCAATCCAACACCCCTATCGTGGGAACCACT  
GGAGCTGAAAGAAGGACCCCAAGGGCCCCCAGCCGCTGTAATCCTTG  
GGGGCCTCTGCCCAGGTGCCAGGTCTCGGGCAGGAGGGGCCGCGGGCACA  
GCCGTGGCAGATGCGCCCCCAAGCCTGGGCTCGGAGGAGCCCCGCCCCC  
ACTGACATTTCCAGGCCGCCCGCTGCAGACCCGGCTGGCCGTGATATTTA  
GACAGGGCTTATTTGCCGTGACTGGTTTTTGTATGACTTTGGGGCCAGGA  
TGAGCTCAGCCGAGCCCGGCTTGGCCACCTTGGTCTCAGCTTGGGTTTG  
ATAATATACGCGTTCACCTGAACCGCTGACGCCCTGCGTGGGCCGAGGCC

Contig 98 (1354 bp)

GCTTGCAGTAGTTTCATCAGATTGGACGACTCATAAATGTCAAGACATCTA  
AAGATTGCTGCATCCAATCATTCCCACCAGTTGTTTTTTGTAGATGT  
CAAGAAGCTGACCCAAAACCTACGTGGAAATGCACGCAACTGGGAGAG  
TTGAAACAATTTCTAAAAAGAAGAAGGACGTCGTGGGAGGACTCTTCGCG  
CTCTTTGGTTTTCGCTTCACTTTATATTATTAGTACTGATTTTCTAAAA  
GCTCGAGTAGTCAGACAGACTGGGCTCTATGAAAGGAGGGGCTCAGAGAT  
GGTTGGGACAGAATAGAAAGCCAGAAACGGACCCCGCAATGTGGTCA  
ATTGAGTTTGGGCAAGGATGTGAAAGCGGTTCA GTGGAGAAGAGTCTTTT  
CAAGAAATCTCTGGTCTGGATCCACTGCTCATCCAGGCCAAGAGTGAA  
CTTGGCGCACATTTCTCAGTGTATACAAAACCTGACTCAAATAATTC  
ACATAACCTCGTGTAGCGTATGAAGCCATGAACACTCAGAAGAAAATCT  
CGGTAAACCTCAGGGCATCTGGGCTCCACCCTCAGACCACTGGCCTTG  
GGGCCAGATACTTACGTGTTCTCCTGTGCACTGTGGGACGTGCAGCCAAA  
CCCCACAAGGTGACCATCAGAAATGTCTCCAGACGTGCGCAAATAACTG  
CCAGAGAGCAGAGGAGCCCTCACTGAGAACCACAGGTTGGGGCAGAGAG  
ATCTCAGACATGACACGATTAGGGGAAAACAATCTGACACACTGGCTTTG  
TTAAATTTAAAATTTTCCCTGTAAAGGCAATGTAAGACATTAAGAG  
GCGAAGTGGCAGACTGGGAGAAAAATTTTGCAATCATGTATCAGATACG

FIGURE 6, CONTD.

AAGAAGATGCAGGAAATCCTCAAAGTTCAGTCACAAGAAAACCCAATTCA  
 AAAACCAGCAGAGCAGACATACGATGGCAAATAACCACGAGAAAGTCAGC  
 ACCCGCTGTCCCTGGGGGGACGCGAGTCAAAGCCAGGAGGACACCAGGAT  
 ATGCCCACTGCCAAGGCTACGGATAACGGGAAGCAAGAGACACAGACAGA  
 AAGGATGCTTCGGTGCTGGGGAGGGTGGGGTGGGGCGGGGGTCCCCC  
 TGGAGCAGGATGTGAAGGCACCTGGGGGGGGCTCTGCACTCCTGGGGGCC  
 TTTGGCACAGGCGGAGGGCCCGGAAGGCTCTAGGGGCACGGAGAGGGGT  
 GCCAGGCTTCCTTACCCAGCCAGGCAGACCAGGCCCTGTATGAAGCCT  
 GACGTGCAGCAGCAAGAGCAACATGCTACAGACATGTGTCTGTGTGTG  
 TGTG

## Contig 99 (1000 bp)

GGTTCCTCAGGCGCACGGGCGAGAGGCTGAGGGTCCGAGGGGCTTTGGGTG  
 CTGGAAAGCCTGAGTTGAATCCCAGCTCGGTTTCTTAAAGCTGTGTCTC  
 CACGGCCAAGGAATGGGGCCTCTCTGGGAAAGGTCTGGGGTGAGGCTGGC  
 GGGACCTGCCAGCCCCGAGGGCATCTGACCAGACAGTTCTCAAGCTCA  
 CAGGGCTTCATGGCAGGATGGGGAAGGCTGTGGTGGGGAGTGGGGAGCAC  
 TCGACACCCCTCTCCAGGCCTCTTGAGTCACGGTGGCCTCTGAAAAGGGT  
 TCTCTGTGTCCAATGAGCAAGTCTTTGTCCGGGGCAGGATTACTAAGTCC  
 AAGGGTGTCTGCCCCCTCCGTGGGGCACAGAGCAGGGGGCCAGATCACGT  
 GGCTGTAAGTCCAGGTTGCAAAGCCTGCCACCATGTCCCACTGGGTCT  
 CCAGTTACCTTGGGAGGTGCAGGGTGGGGTGATGGGGAAGTGAAGCAGA  
 GAGCTGGCAAAGAGTGCAGGCGAGGACTGCGGGCGCCAGACCCAGCTAA  
 CCGACCCCTCACACGGAGCTGCTTCTACTTTGCAGCCTGGACGTGGGAAAA  
 GGTTACCCACACAGCAGCGTGTGCAGGCACGCTGGTATGTCTGTGTACTTA  
 TGCATATGTTCTACGTGCATGCACGTGAGTGTGCTGTGTGCATTGTGCCT  
 GTGTGTGTGTGCATGTGTGTGTGCACTCATGTGTCTATACGTGTGTGTAG  
 TGAATGCTTGTGCATGTGTATTTGCATGTGTATGTTGTACGTGTGCAGT  
 GAATGCATGTGTGTGCAGTGGCGGCATGTGCGTGTGTGCGCATGTGTCTG  
 TTTATACCTGTGTGTAGTGAATGCATGTGCATGTGTGTGTTACATGTGC  
 ACGTGAGAATGTGCACTCGTGCATGTTTGCATGTGACTTTTCATGTACACA  
 TGCTTTTAAAGTGTGCACGTGTGCACATGTGTTTCTGTGTCCCTTGCACG

## Contig 100 (1500 bp)

CGTATAAATATATTAATATAGAATAAAATAGATTGATAATATAGATAAAC  
 TAAACCCATTATCAATACCGGGTGGCCCCAGCAAAGGATACTAGCCAGTT  
 TATCAAGGTGCTAAGTCAGCACATAGAATGGCCACAAACGAAAACCTGTA  
 CTGCCTATGTCCACTCTAATGGAGTATGCCACTGACATCAGTGGTAGGTG  
 AGCTGAGTCCATCTGGGCTCCAGTTCGGGGCCCGCTTGTCCCCAACGG  
 AGGTTCCCTCCAGGGTTCCCCAAACCAACCGGGCCCCAGGTCTCCCTG  
 TCTTGACTCGTTTCTGGAGTCTTCTGGGGCTCTGCAGTCTCCCTTGTG  
 GGGCTTCTGTCCCCCTGCCCCTGGCCTTGCAGGCTCGGCCCTGCCCTGGG  
 TCCCGGGCCTGCGGGCTCACCTCCTTCTTCCCTGGAAGAGAGGGAGCC  
 AGGCTGGGCGGGCCAGGAGGGAATGCGCCTGACTCTGCTCCAGATGGAC  
 AGGTCGGGACATGCAGTGGCCTCGCCTTGGGCTGCTGAGCCAAGAGCAGG  
 ACGGGTCTTTCTGGAATGTGGGGCCAGCCAGGTTACGCGTGTGGGTGGG  
 CAGCCGCCAGCATCTGTACAGGCCGCTGCAGGCGCGGGGAATGACCTCGA  
 CTTCTGCTTGGCACCCAGCTCTGGAACAGCCCCCTGCGGAGCCTCCGCCC  
 AGAGCTGGGCCAGAGGTCCCCTGTGCCGGGGACCCAGCAGGGCCCCCTC  
 CCTGACTCTCCAACCCACCTGCCTGGGAGGAGTGGCCCCCTGGCCTCCGT  
 GGATCTCTGGGTGCGGGCTCAGCCGGCTTGACAGCCTGGGAACAGCCAAT  
 GCACATCCCCAGGCCTGGCCACACCCTCCACCGGGAGCGGGCGGATCTG  
 CATTTCGCCAGGCTCTGCGGGCAGCTCTGAGAGCCCCGGGTCTCGGAGCC  
 CAGCCGTGGCCGTTGTACGCCCTGGGGGCTGTGGACAGCGTGTCTCATT  
 GCCCCCTCCGAGGTCCGGGCCAGGTCCCTCCCACTGCTCGCCCAAGAGCC  
 CTCTCCCCACCAACCACACTTCTGTGTTCTGCAAGCGGGACACACACT  
 CCGGTTTCAGGACCTTGTACAGTGCCTTCTCTGCAGAGAAATGCCTG  
 GAGCAGATGTTTGTCCGCACGGCTGCTCCGCGAGGCCTACCGAGAGCCCC  
 TCACCTAAACGGCCGGGCTCAGCAGCCCCGGGGCCCTGTCCCCACCGCCC  
 AGGTGGTGGGTTCTCTGTGCCAGTGTGGGCATCTCTGTAAGATACCTGT  
 TTATCTGCTCATCGTCTGGTCTCCCCCAGAAGGTAGAGCAGGGCCCCGCA  
 CAGCCGTCCTCGGGGTGGCCACTCGCCCTTGGGGCTCAGCCTCCATGCAG  
 GGAGGGACGCCTGGTGACACGAGAGCCCCGTGTGAGTGTGCGGGCGGCC  
 AGCCTGCCTTAGGTACAGCCAAAGCCGGCATTAAACCACAGGCCCTCGA

FIGURE 6, CONTD.

## Contig 101 (600 bp)

TCTAGAATACCTGGCCCTCCAGGGACGTGTCTGTAGCTGCGGCTTTCAG  
GGCAAAGTGTAATTAACATCCCCAGGCTTCCCTTCCAGTTGGCACAGGG  
CACCCACATGAGGAGCAGCCTCTGGGTGCCAAAGGGCCCACTGGTGCCAG  
GCGCTGGGCTGAGTGACCCCCCGCATGCTTCCCGCCCACTCACCTGCTGG  
CCCCACCCCTGACCACAGCACCTGTGGGAACACTAGGCCTGGCAGCCACA  
CGTGTCTCTCACTGGAGGCCAGTGCCAGGCAGCCTGCTTGGCTACGCTAG  
CAGATGCCCCGCTCGCCTCTGCCCCCTGCCCCCTAGCCCATGCAGGAGCCAG  
GGTGGGGCACAGGAAGGACGATTGGGGCCCCAGGTACGGCACATCCAGGC  
CACAGCCGTGGCCACACGAAGGCGGCCCTGAGGGGGCGTTGGGGGGCAGA  
CCCTGCCCCCCCCGCTGCCCCCAGCTCCAGGCATTAATCCCAGGGACC  
TGTTGCACTGGGTGGCCGCGCAGCCTGCCCCCTTGCTTCCAAGGCCTCTA  
AAATGCCCTCTTTTCGTAAACTAGGACTTACCAAGCTCAGCGAGCCCTC

## Contig 102 (1867 bp)

AGTATATCGGGTGAGACTGGGGACCGGTCTGCCGGAAGCCCCACCATAA  
AGGCCACGTTGGGCCACAGTCCGGGCCACGTGAGTGTGGGCGGTCCGCG  
GGTCTGCTCTTGGAACACCAGGATCTCTAAGAGGTACCAGCCGAGGCCAA  
GTTACAGTGAGCAAGTGAGCAAATGACTGAATGAGAGCGTGAGCGAATGA  
GTGAGGGGTGAGTCCGTCCACCACGAGCCTAGGCTCAGCCAACCGCTGT  
CCCCGCGTCTCCACTGGTGACCAGAACGAAAGAGTGGGGAAAGAGTGGT  
TGCTCTCCACAACCCAGTCCCCAACCCCCCTGGACGCCCCACCCCTCCAG  
GGGTGCCGGGCTGGCCTGTGGGCCCACTGTGGAGGCTCTGGCACCTTC  
CTCATCCGTTCTCCAGCACCCAGGTTCTGTGCTGAGCCCTCTGGCCCA  
CAGGCCTCGGGGACAAAGAGGGCCACCTGGAGGCTCAGGGAGCCTCACCT  
GCCTCGTGGTCTGGCGGAGGCGGTCTGGACATGTGATAGACCGGCCTG  
GGCTCAGCAGCTCCTGCTGGAAGATGTCAGGGACAGCCTGGGCCACTCTC  
CCACCAGGAGAACTTATTCCTCGGTGGGGTCCCCCGGGGAAGGGATGGG  
ATCCAGCGGGGACCCAGAGCGTCCAGCACACGACCTGTCCCTCCAGC  
CCCTGCCCCACACGGATGCTCACAGCTCAGCCTCGAACACGCACCTGTTG  
GACTTTGCTCTGAGGCTGTCTTCTCAGCCGACGCGGGCTCCGCTGCA  
TGGTCTGGAAGCCAGTGGGACTCGGTGGTGACAGGGAACAGGGGCTCTT  
GGAGTGGGGTGGCGGGGAGCCCCGAGGGAGCTGCTTGGGCCTTTGATGG  
CTGAGTGGGTGGAAGTCAAGTCAGGCAGGCTCCCCCAGGGCTCCCTGACCCCCC  
CACCTCAAAAAATCCAGAGCATCCTTTGCTTTGGGTCTGGTGAGGCTCTC  
TGAGGTGAGACCTGCGTGGCTGGGCCAGTGGGGCTGGAGCAGGAAGAAA  
GCAGGACAGCCCCCGCCCTGGCCAGACTCCCCAAACCCAGCAGGAGAC  
ACCTGAAACGGGATGGAACCATCCTGAAAAGAGCCACCTCCTCCTCTTA  
TGCATCAGCTGCCGGGTCTGGGGGCCCGCCCCAGGCCCCAGATGTCCGG  
GCTGCTCCCGTCTCACATCCAGGGGTTTCTGGGGCCAGGACTCTGTCCCC  
CCAAGCATGCAGAGGGTCCAGGCTGGGGTCTTCATGCTGCCGTGTGCA  
TGGTGGGGAAGGAAGGGGACAGTCTGGAGACCCCCGCGCTCCCATGCG  
TGGCGCGGGGGACAAAGCCGGTGGGGTCTCAGGTTTGGGTTAGAGCA  
AACGTTGATCTGACCTGGTTCTGAGATGCTCGGCCGATGCTGCGTTGTC  
CGCTCGCATTTTCTGTTTCTCTGGGAGGCGCTGCGTGCGCTGTGGCTT  
CCGGCCAGCCCCACGAGGGACGAGGGTGGCTGGCGGGTCTGGGGGCC  
CCTGCCCCGACCAAGCTCTGGCTCAGGTTTTGTCTCGTGACCCATC  
ACTAAGGGCCACCTCTGACCCGAGCCCTGTCTCCGAGGTGGGAATTGG  
GGCTGTCCCTGGCGTCATAGGACCTGGTTGGGGGCATCCAGGCTGTGT  
CATGCCCTCCCCAGAAGACTCTGGGGGCTGCGGGAGGGTTTCCCCAGCT  
TCGGGCCAGCCTGGGGAGGGCGAAGGCGCTGGAGGCCTTGCTGTCCCA  
GGGAGCATGGCTTCGCTGCAGACTGGGGCCCCGCACACCCAGCCACCACT  
GGCCGTCTGGAAGCACT

## Contig 103 (650 bp)

GTTGAGGATTCTCGGCAATTTCTCTGCTCACTGGCGCTCCAATCGCCTCG  
ATGGGCTTCTCTCCAGATACAGCTGCAGATCCTGGGCGGGCACACCGTT  
GAGCGTCACCTCGTAGTGAGATTGCACTCGTTGTCAATGGACATCCAGG  
CCATGCCGACGGCATGTGGATTCTGTGCATCCGTGTGCTCCTGTGCTTC  
AGCAGAATGGGTTCCGCCGAGTCCCGAGCATCGGCCACTGGACGGGGCAC  
TAGGCGGGCCAGGATCAGGCTCGTCTCATGCTCGGTGGCCACATTAACGC  
CCAGTTTCGCCGGCATAACAGGACTCGAGGACCTTGGGACCCAACTTCTCC  
ACACTACCAATGGCCTGGTTGAAGTTGAAGCTCGGCGTCAGATCCTCCAG  
CTTGGCCTTCCGCTGCCCCTGCTCTCAATCAAAGTATGTTGGGGCTAT  
CCCGGGTGTTCACGTGCTCCGTTTCGATGTTGTAGGCCAGAGATCCATCG  
GTGTTCAAGTAGACCCACGCCAAACCGCTGCTTGGTCGAGGATTCCGGC

FIGURE 6, CONTD.

ACTGTGCGGGCGCCAGCAGGGTCTGGAAGATTTTCGCAGCTGGCTCGGGTCA  
CGATGTGTCCCTGGATGCGCAGATGTGGGTACTTCTTGGACTCCACGGTC

Contig 104 (1630 bp)

GGTGTGTCTACTGCTGTGGCTCAGACCCCTGCTGTGGCACAGGGTCCATC  
CTTAGCCCAGAACTTGCACATGCCACAGGTGCAGCCAAAAGAAAATTCT  
TACTAATAAGTTGTTTCAATTTGCCTTTACGTAGAGTGGCATCAAACAGCAA  
ATTTAAAACACCATCTATCAATACATAGACCGCGGTCAAAGGGAAAGAAC  
TTTCTATTTTACGACCTTTAATCATGGCTTTGCCGAATTTGGGACCAGGG  
TGCTGTGTTTTTCTCTCTCCCTGCAGGTGGTCCCGAGATGACCAGGCCGG  
TCCTGGGCGGGAGGAGCCGGACTGTGGATCCAGTTGCTTCCCAAGACAGG  
CTGACAGGAGAGCAGCAAGGGCCACCCCAACCGAAACCAAGCCAGAAC  
GAGCAGAAAGATGCCGTCTTCCAAGTGGGGGCTGGGAGCTTCCCTCCATC  
CTCCGGAGCCGTGAGGCTGCCCTGGAGCTGGCAGGAGCCACAGAGGACCC  
GGCTTTGACCGCCCTCTGGGACCCACAATCAGGACCCTGACTCAGATGC  
TGAGGGGCTGGACAACACCCAGGACCCTGTGCTTCCCCAGAACCGCT  
GTGTCCATCAAGGTCCAGATGGCACCCGTGTCCCACTGGAGCACGCACT  
CCGTGGGGCAGGCTTTCCCTTGGGCACCGATGCACCTTGAAGGACAGAGAC  
GGGGCCCAATAAACGTTTCCAACACAGTGGGTGAGGGACCCGACCGGCC  
GACACGGCAGCCCGATGCAGGGACTCCGTGCTTGGCCAGCCTCCCTTG  
GGGTGGTCTGTGTCTCTCAGGGGTGGATAGGCCATCATGTGGGTGGCCTC  
TGGGGACATCCGTTCTCTGATTGGGTGAGTTTCAGCCACAGAGATATTCC  
CAGGACTACAAAGCTGGGTCCCTTGGGGCACCTGCTGTACAAAAAGACA  
AGGCCCTGACCCCAAGTAGCCAAGTTCCCCCAGGGGCTCCCCAGGGTCTG  
GTCATCCAGACTGTGCCAGCCGTGCTGCCCCCCCCAGTCTGCCTGACCC  
GAGTCTCTGTAAACATCCCCGGCCCCACCCAGCTTTACCCCAAGGCCGA  
AAGCACCAGCCCCCTGCACCACAGATGAGGCCCCCATGGTCCCCGACC  
TAACCTCTGTCTGCAGTTGGCTTTACGCTCGGGTGGGGGCAAGGCCTGC  
ATCTCAGGCTCCCCGGGAGAAGTTGCTGCCTCCACAGCAGAGCCAGGGCC  
TGCTGACCACCTGGGCGGGTGGATCTGGTCTAGAATGCTGCTAAGGTG  
TCCTTGACAGGACGCCCCGGGCGGCCCTCCAGGAAGGAAGGGGACA  
TTGCCAGGACTCAGGAATGAAGCCATCCAGGTTTTGAATCCCCGGTCCC  
ACCACCTTCCACCTCTGACCTCAGGCACCTCGGCTTTTACAGAGCTGCCCTT  
TCTGACTCTGGGACACGGGGCTGTGAGGCGCTCTCGGTGTGTGACAGCTG  
GGGGGGGGCACTCTTAACGAGGGTGGGCGTGCCAGGTGACTGACCACA  
GCCCTTTCCTCTCTCAAAAACGCCCGCCGAGTGACCTCACGGGAGGCAG  
GGCCAGGAACCCCAACCAACAGAAATCA

Contig 105 (1820 bp)

AGTGAGCCCTGCAGGACAGTCTGCTGAGGGGTGTCTGGGCTCCTCAGAGG  
CTCATGGCCACGGGCACTGGGAGGATAGCAGGTGGACCCCTGCATCCAGG  
TCCCAGGTCCCAGGTCCCAGACCCCGGACAGGCTTTCTATCTGCAGGAG  
GGGGGCTCCTGGGGCAGCAGGGATGTGGCTGTGAGGCCCTCGTCACTCTCC  
CTGTTTCTATCTCTCTGTATCACACACACACACACACACACACACA  
CACACACACACGACGACGACACACACACAGAGGCGTGACCAGGGCTGCA  
GACAGGGCCATGGGAGGACTGCCCGCAGTGCACCCAGATGGCCACACGG  
TGGGGCCCTCGTCCCACTTTTGCTGCTGATGCTTCCGCCCAGGCTGCTGG  
GAGCAAGCACTAGCTTCCCAGGGCTTGACCAGAGAGGGATGGGAGGGGT  
CATGGGTCAACAGGCGCCAGGGAATGGGGAATAGGATCTGAGGGGCGGGG  
GCAAGGGGGCCAGGCGAGGCTGCAGTGCCAGAGCTCCCTGCACCTGCAG  
GACCAGCCACAGGCCAACAGCTGCAGGCAGAGCAGGGCTGCTCCTGTCCC  
CAGAAGCTGGCACAGCATGGGGTCTGACAGCCCCACCCGGGCTCCC  
ACAGAGGGGCGGGTCCCCAAACTCCTCCCCGTCCACCTCACAGCTCA  
GCATCTCCACTGCCTGAGGACGAGCCCAACACACGGGCACACACACAT  
GCACGCACACATGAATGCACCTGCAAGCACACACTCACACGTAAGCAG  
GTACACACATGCATGCACACAATGAACACACATGCACGCACACACGCATG  
CACACACGCACACACACTCAAACACGTACATGCAAGCACATGCTGGTCTT  
TTGTCCCCGTGGAGGGGAGGATGGAGGCCAGCCGTGGGGAGGGCATGT  
GGAGTGTGGGGGGCTGGCTCCAACGCCCTCGCTCAACAGGCACCAACGC  
TGGACTGAGATAAGCCGGGGCGCTGGCTCCCTTGGGGCCGCTCAGCAGGT  
TTGACGCCCCACAGGTGGCACTGCCTCTTTTCAAGACGGATGTGGCC  
ATGCCACCCCTCACAGCCTCACCAGTCCCCCTCAGCTTTAGTGGTGTCCC  
TGTCAGTGTACCCGGGGCTTCCCTTCTTCCAGGGCCAAAAGCGAGTTTCA  
GGGACAGTGGCGCCCCATAATTACTACCCAGGGTGTGCTCTCTGTGG  
TGGCCTTGAGGCCAAGGTGCTCCCATGGGGGCCACAGGGCTGGCAGGGT  
CACTTCCTGAGAGCACCAGGGCCAGGGGGTGGCCAGGCCTGGCCGGT

FIGURE 6, CONTD.

CCCCATCTGGAATGAGGGCCTTGCGCAGAGGCGGTGCACCCCTCTTTACA  
GCAGCCCCGGGGAGAGTGACTCCTGCGTCATGGACCTGGGGGCTGACCT  
GTCACGTGTCTCGCCAGTTGCACCCCATCCATTTCCGGGTGGAAGGGAC  
AAAGCCATCCTGGTCTCTCAGAGGACCTCTGGAGCCTCTTGGCCCCAGC  
AGCCCAGCCCTCCCGGGCCCGCATCCTCTGCCACCCAAAATCACCTGT  
GCCCACAGGGTCCCCTTCTGGGTGTCCAGGGCGACCCAGAACTGCCCTG  
CAGACACACCCAGCCAGGACATGGCCGCCCTTGCCGGGCTGTCTGCCTG  
GGGCAGCCTGACTGCCACAGACAGGCCGCTTGGAGGACCATCTGCCTGAG  
CCCCCAAGGCACATCCCACGGGGCCACACAGCCAGCGCCTGTAGACGAT  
GCCACTTGGGGTGGGGGAG

Contig 106 (1500 bp)

TGCCGAATAGAGGTGGAACCAAGACCCGAAAAATGTCCACATTTTCA  
ATTATTAGAAATTTAGAAAAATATTTTACAGGAGTTAAAAGGTATTCCAT  
TCTGGGGGCGGGTGGGCATGCCACGGCATGCAGGCATTCCCCGACCAGC  
GACTGAACTCGAGCCACGGCAGTCACCATGCTGGATCCTTAACCTGCTGA  
GCCCCCTGGGCAACTCCAGACACTCCATATTCATGTAACTATTTTTTAAC  
CAAAAAATGACAAAGCTTTTCAAAACAAAACACATTTTATGGGAAGAGT  
GGCATTTGCTTTCAGCCTGGATGGTCTGCTGCGGCTTGCGGGACGACGAGGG  
CCCCCGCGGGAGCGCCTCCGCACGGCGCATCAGGACGTGGTGTCCAGGGA  
AGCGGGGTCACTTCACGGCTCTCGGGTGCCTGCTGGGTTTCTTTTCGGC  
ACCACACCCGGACTCAGCACTTGGGGGTCTTAAACGTGAGAGGCACTGC  
GGGGCTCGAAGCCACATCACTGACCTCCTCAGACTCTGTTATGTGAAAAC  
CCATCCGTCACGAGACCAAGAGACAGACGAAACGCAAGGTGGCGC  
CTAGGTTGGGCACAGCATGAGGGCAGAGCGGAAACCTTGGCGAAATCCCG  
GCGAAGCCTGGACGTGCCAGCTCTTACTTGACGCAACATAGGGGGATT  
CAGGAACCTCTTTTACCGCATTTGCAATTAATTTGCTGCAAACTCTAAAT  
CGTTCCAAGCACAATGCTCACTGCATGAAAAACCCAGGGGTAGGTCTCG  
CCCGATCAGGATGTTTTCCCGTCCCTCTGTGCGGGTGTGCCCCCTGCG  
CTGGTCAGTGAGAAGTGTCCCTCCACCGACGACATGAACTTCCCAGGTG  
CACGCTCTCTGCTGTCTTGACGAAAACTCATCTCTGTGAATCTCCCGCC  
AGCTCCGCGGGAGCCTTCCAGGGCTGGAAGGACGGCCGTCCCGTTCAGG  
GGGCAGGTGCACGCTTCCCAAAGCTCCCGCTCTGCTAGGACGCTCAGAC  
GGCATCACCCACAAACCCACGAAGTGTTCCTCGAGGCGACAGGCTCG  
CCCTTCTCCGAGAAAGCAGCCCGCACACGTCAGCAAGGGGGCAGCTGCGT  
TTGTAACCTCAAATGGCCACATAGAGTTTGTCTTGAGGACAGGGGTCTGT  
CTGGGCCGACCACTGCACACGAGAATATGCTGGGACACGCTCCGGGGT  
CCAGCTTTCATGGAATTAATAAGTTTACTGCTTCACCAAGTACATTCTTA  
AGTGTAGCTGGCCGCCAGCCTGGGCGTCCGCTCCGAGGCTGCCTCTCTGC  
CTGGAACCCCTTGTGCTGGGGGACCCTCTCTCCAGCCCCACCCAGCCCCG  
AGCCCAGGCAACATCCTTCTTGTAAAGACACCCGCTACCCCTGCCCTCCCGC  
TTCTCCTTCTCTGGATCCAATCTCCTCCGCTTCTAAGCTCTCTTGAGGCT

Contig 107 (550 bp)

ATGGCACTCGCGGTTGTGACTGAGCTACCGGACGGCGCGAGCAGGGCCAC  
GAGGGCGACAAGCGGGGGCTGAGAACCTGTGCGAGGGCAGGTCCCTGCG  
GCTGCAGACAAGCCTCTATCGCAGGCCCACAGACAGGAGCCCCGTGTGA  
CCCTCAGGCTGCGAGACCAAGTCACGGCTCTGCTGGGAAAACCTCGAAC  
CTGATGACTGGGTGGGTGACCCAGGACCTTGAATCCGGCCTCTGCAGA  
ACGCTCTGAGCCTACGGGAGTGGCCACCCTCTCGGTTAGGGCCTGTGTCC  
TTCCCTGGCTTCCAGCCTAGAGCAAAAGCATTAAATCACAGTGTGGCCCA  
GCCCCGACCGTGCAGGACCTTAGACAAAAGAGGAGGAGAGAGATGAG  
GCAGAGAGGCGAGAGACAGAGGTGGAGAGACAGATAGACAGAGACAGAG  
GCAGAGAGAGAGACAGACAGACAGAGACAGAGGCGGAGAGACAGACAGAG  
ACAGAGGTGGAGAGACAGGCAGACAGAGACAGAGGCCGAGAGAGAGACAG  
Contig 108 (900 bp)

TTTCTAAACTCTCTTACTAGTTCTAGTTTTCTATTGTTTTCTGGGGGGT  
TCTATATAACATTCGTGTCGTGATTGGAGATGGTTTTGTTTTTCTCTCT  
CCAAACTGTATGCCATGTGTTTTCTTTTCTTGTCTTATCAGACTGGCTAG  
GACTTCCAGTAAACACTAGATATGAACAATGAGAGGAGAGCCAGGCCCTT  
CTTCTCAGTCTTGGAGGAAACAGTCAGTCTTTCTCATTAGAAATGAGAG  
CTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTTCTTTT  
AAGGAACCTCTCTGTATTCTTATTTTTTTAGAGTTGTATTTTTTTTTT  
CTCTCTTTTTTAGGGCTGCACCCGAGGCATATGGAGGTTCTAAGGCTGGGG  
TCGAATTGGAGCTACAGTCGATGGCCTACGCCACAGCAATGTGAGATCTG  
AGCCACATCTGCGACCTATACCACAGCTCACAGCAATGTCAGATGGTTAA

FIGURE 6, CONTD.

CCCCTGAACAAGGCCAGGGATTGAGCCCGCATCCTCATGGATGCCAGTC  
AGTTTCGTGACCGCTGAGCCATGAAGGGAACCTCCAATAATGCACCAATT  
TTAAATGAAAAAGACAAAGCATCCAGCCACAGCCTGAGTAAGGAGTTTG  
GAGGCCCTGACCCCTGCGTGGTCTGGGCTGGGCTGGTCCGGGT  
GGGGGGGGTGGGGGGACCCCTGTGGACCCTCCCTCCTCAGCCAGGCCCTG  
CCCCCTCCATCCCTAGCTGTGGGGGCTCGGAGGAAGCGGGTGGATGACG  
GTCCCTGGGACCCCTCCTCATATGTATCTGGGTCCCTGGTCCCTCTGAGG  
CCCAGGTCAGGTCATGGGAGTCAAAGGTCAGCCAAGGGGGTAGCCCAGAG  
Contig 109 (950 bp)

TAACCCACTGACCGAGGCCAGGGATCAAACCTGCAACCTCATGCTTCCTA  
GTCGGTTCCGTAACCACTGCGCCACAACGGGAACTCCTTTGCTTTTGT  
TTAGGATTTACATACAGTGATAACGTGCCGTATTTATCTTTCTCATCT  
GAATTATTTCACTTAGCCTAAGCCCTTCAGGGTCCATCCATGGTGTGGG  
AGTGGCAGGATTTGCTTCTTTTTTTTTTTTTTTTGTGGCTGAAATCAG  
TCCAGGATTATCTTTCTTTCTGTTTCATCTGTGGAGGACACAGGCTGCGT  
CCGTGTGACGCTCTGCCGGGAATACGGGGGCCGATCGCTTTCTGAGCCAG  
TGTTCTCATTTTCTGGGAGAAGTACCCGGAGTGGAACGGCTGGGTGCTC  
CTGCAGTTCTGTGCTGCATTTTTTTGAAGACGCTCGGAGCGCTTTCCACAG  
TGGCTGCACCGACTGACATTTCCACCGAAGTGACAGGATTTCCCCATCCT  
TTTTCCACGTTTTCCCGCACTTGCTATTTTTTGCCTGTGGATGTCCGGCC  
TCTCCGTGAGGTGTGAGGGGAGTCTCCGTGCGGCCAGGCGAGGAGCGAC  
CGTGAGCGTCTTTACGTTTCTGTGGGCCACCTGCGTGGCTTCTCCGG  
AAAAAGGGCTGTTCAAGGCTTCTTGCCCATTTCTCAGTCTGATTGTTGGG  
GGGTTTGTGTTGAGTTGTGTGAGTTCCGCACGTATGGGGGGCATCAACC  
CTTTATCAGCTATGCGATTGGCAAGTCCGTTCTCCCATGTTCCGCCGGCC  
GCCTTGGCACGTTGTGGGCGGTCTCCTTGGCTCTTCTTGGTGCAGAAGGC  
TTCGGTCTGATGTGGGCCCATTTGTTTATCTTCTTTCTTCTCACCCT  
TGTTTTGATGTGAGATGCAAAAATCCATTGCCAGGGTCTGTGCCGAGAAC  
Contig 110 (306 bp)

CGCCACCTCAATCGCCGGTTTGTCTGCAACACGGTCCAGATAACCAGCG  
CACCTAACAGGTGCAACACTGCCAGAAGTGCAGACAGCGGGCTGAAGCCG  
ATGGTGTGAGCCAGTGCACCGACAACAGCGCAACAGCGTACTTGCCAG  
CCATGCCGACATCCCGGTTAAACCGTTTGCCGTTGCCACTTCGTTACGAC  
CAAACACATCGGAAGAGAGCGTAATCAGCGCGCCAGACAGTGCCTGGTGG  
GCAAAACCACCGATACACAGCAGCATAATTGCGACATACGGGTTGGTGAA  
CAGGCC

Contig 111 (800 bp)

GTTTCCATGATGCACCAGGGGGGCGGGACCGCAGCAGGGAAGGCTCCA  
TCTTGGCTCTGTAAGACCTTGAAAACACCTCATTCCTCTGCTTGGCCT  
GCTCTTCGGTACGCCAAGTTGCTGAGACTGATGTGGGGATCAGTGGGGAG  
CAGGAATCTTTCTGATTCAGCCGTTTCAAAGTGTCCCAAGCAGAAGCTGT  
GATGGCAATGCCAAGGCTATCCATGGAGGTGCTGTGCCAGGGGCCCCAT  
TTCCTGGGAGCCCATTCAGGAAAGGAATCTTGTAGCCCCAGGCTCCAGC  
AGCCAGTGCACGGCCCTGGGACTATCCGGGTAGATCAGAGGGAGGAACA  
GAGCTGTGGATGGTAAGCAGGTGGCCCAAGTCCAATTTATGTCTGTGGT  
CCAGCAGGGTGGCCAGGAGGCCCTCGTAACTCTTAAGAATCTTGGTCTG  
GTCAGCTAAATTGTATGACCATTGTACTGAGCACACATCCCGTTAAGTA  
GAATTTTCAAGGATGACTAGGAGTTTGCACCTGAAGGCAGGAAGGGCAT  
TCCAGGCAGAGGGTACAGAGGTGAGAGGGAGGCTCTGACACTTTGGGCGT  
GCAGGGGTTTGTGTTGCTGCTGAGTGGCAGTGGCAGCAGTGTATGCCAGGCCT  
GGCAGGGCTGTGTTGGTGTGTTGGAGAGGAAGGGAGAGGTGAGTTGAGCCC  
AAGGTCTTCCAGGCCAAAAGACTGAAGGTGACCGCGGCTGTCCGGGGCTG  
GCCCCAGACCAGGAGGGAGCAGGTGGGAGCTGGCTCTTGTTCGGGGAC  
Contig 112 (3062 bp)

CACACCCAGGAGAGGAAAGACCCACACAGTCTGATGACAGCTTGGCTC  
GGGGCTGGAGCCCCGAGTTATAAATGTCCATCACGAGCTGTGTTCTGTCA  
GAGCCATCAGTGGGAAGGCCAGGCCAGCTCAGCAGCCCCAAAATGAAGAG  
CTAGGTCTGGGATTGGGCCCAAGCAGAGGGCACAGGAAAGCCACATAAAC  
AAGGCACCAACCCCCCTGTATCCACCAATGTACATTCAAGGTACACCC  
CCTGGTCTTCGGGGGAGGTCCCCTAAGATCCGGTGGCAGGGGGAGGAAAA  
GTCTGACTGGATTCTTGACAGGTGTATCAGCGGAAGGCCAGGAGGAGTG  
CTCGGGCACTGCCACCTCCAGGGGCATGATGGTGTATGGACAGATGGCA  
GTTATGGGAGGAACCTCCCCGTGGTCAGAGCTCTGGGTGCTGTACCTGG  
TCATGCATTTGAGTGAAGGAAAAGAAAACATACAACCTCCACCCCCAGC



FIGURE 6, CONTD.

AGCTTTAGGCTGTTGGTCTAAAGGTCCTGCCTCCTGGAAGAGACACGCCT  
CTGTGACGGGACACTGCTAAACCTAAAGGAAGAAGTGCACCTGGTCACG  
GGACTTCCTAGGCCAACCAACCTACAGGTGACGGCCCGGAGCATCACGAG  
GAGGTAGGGGACGGGAAGGGATGCATTTGCTGCTCAGCGGATCCACTGGG  
CGCTTTCTGGAGCCCCACGCCACACTTTACTGCAATGCACAAGCCCC  
AGGCAGCAGGACAAGTCACAGTAGCTCTGGGTATCCAAGGAGTCAGGGA  
CCTACCTGGAAGAGTCTAGAACAGGTGACAGAGGAGGAGAGGATGGTAC  
CAGCAGTATAGGGAGAATCAGAAATCTGACCCACCCCTGGGGGCTGACTG  
ACTCCCAGACCAAATGCCACACTCAGGTTCCCCGTCTGCCTGCACTTCCA  
GGGCTGGGGCCACGGGAGTTATGGGCCCCAGGTAGCATCAGAGGCTCCAG  
GTACAGGCACAAGCAGCAACCACAGGAGGGATCCAGGCCAGGGAGCATCC  
AAGAAGCAGCAGAAGCTCCACCTTAGGTACAGTTCTGGCACCTCCAAGTT  
GAGAACATGTCTTAGCAGTGCCTGACCCCAACCAATGGAGTGTCTGGG  
ACTAGACTAGGCACGCCATTTTGGTCCCAGGTTGCCCATCTGTACAAAG  
GGTGTGCGGCCCCAGGGGGACACAATGAGCTCCCATGGGAAGGGTCTTG  
CGAATCTCCTTAGAAGCAGATGTAAGAGGTGACGTCCAGCTTGTGCCTGG  
GATGTAGAAGTGGAAAAAGCACCCCTCCCCGACAAGGATGAAAGCAAGA  
GGCACAAAACAACTGAAATTTCCAACGCCCTGGAGATCCTTGGAGAAC  
TGGGATTCTCCACCTGTAGGGGCACCTGTGAGGAGAGGCTGTGTGAGCAC  
CTGCTGACCTGGCACAGAGGATGCCAATACTAAGAAGCATCAGCTAAAA  
GTCTCCAGGAATTCCTGGAAGCTGAGGAAGGGCTCAGGAGAGGGTACAGA  
AGCCCTGGGGCTATAGATATAAGGGACGTGCACACCCACTTGCAGGTCCC  
CATGGACCCAGGGACATTACAGTGTGGGCAAGATTCCCAAAATGCAC  
CCCTTGTGTGTGGGCTGGTTCGGTGGGTGAGCAGACACACACCAAGG  
CACAAAGCACACCCCTCAGGCTACTCTCCTCCCTCTCCCTTGTGGAAAC  
TGAGCCTTGAGATGCTGGGGCAGGTGAAAAACACTGTACACTTAGGTCC  
TGGTGAAGAACTGACTGCGGCCAGCGGAAAGAATCATAAAGACCCCTACACC  
CACACACAGCCTTAATTACAGTGTGAGTGGGGCTGGAGCCCCAAGAATG  
TCTACACCCATAAGACATAGCGTTAATCAGAAAAACAAGAACAGCCCCAA  
CCCCACCACCAGGCTGACAATAACAGGTGATGTTGGAATATCACTGGGA  
ATGTTCTAGGAGTGTAGAAAGACACACCAACTAGGGCATGATGCAAAGAT  
AATACTTCAGCCTGGGAGTGGATGTGACACAGGGAAGCATAAAGTGAT  
GGCAGAGGACTTTGATGTGAGTGTGGAAGCCAAAAAATTCTAGCTTA  
GCTCCATTTCCCAACAAGATTGACTGCAACCCCATGCTAAAACAACAGCA  
AAAAGAAAGAATCCTCATTTCCAGGCATAAAATTTTCCCCCAGTCTCTG  
CTGTCTCCATAAGATGTCTGATTTCAACAGGAATTACGAGGCTATAAGA  
AAGGCAAGAAAAAACTACACACTGTCAAGAGAAAGCCATCAGAAATAACCA  
GACTCGTAGCACAGACACTGGAATTGTGAGGATATTTTAAATAACCGTGA  
CAAATACATTAAAGATTCTAATGAGAAGGGGGTAGACATGTAAGATCACA  
TAGATTTTCAGCAAAGAGATGAAACTCGAAGGAAAAATTAATGGGAGCCCT  
AGAGTGAAAAACACTGTAGCAGAGAAGATGGGTTTCATCCGTAAACATGAC  
ACAGCTTAGGAAAGAATCAGTGAAGTGAAGACAGGGCCACAGAAAATAT  
CCAAACTGAAATGCAAGGAGGAAAAATATGAAAGGGGGAGAGAGAAAA  
ATAAAGAACAAGCATCCAAGAGCTGGAGGGTGACACTGAAGAAGAGAG  
CATAGGCATAGCTGGAATCTCAGAAAGAGAGAAAGAAATAACCAAGATG  
TAATGGATGAGAATTTACAGAAAGCGTTGTCAAGCAACAAACCATACATC  
CAAGAAGCTCAGAGAACACCAAGCAAGTAAGTACTGTAAAAAATAGCC  
CGAGGTATACCTCATTCAGGCTGCTGAAAATCCATGACAAAAGAAGTCTT  
GAAAGTAGCCAGAAACAGAAGGCGTGTTCATTGAGAGGGAAAAAGACACC  
ATTGTTGCCAGAAACCAATAAACCAGGGCTGAAAGGGTAAACTTTTTT  
TTTTTTTTTTTTTTTTTGGCCATGCTGTGGCATGTGGAGGTTTCCCGA  
TCAGGGATCAAC

Contig 113 (1300 bp)

AAACGGATAAATACAGGTGACCCACAGGCAGAAGCTGAAGTACAAACAGT  
TCACAACGGCACCACAAAAATACCGAAGGCTCAAGGGTAAATCTGACCCC  
AGATGAAAGGCCTTCTCACGGAATGGCAAAGTGGCGCTGAGAGGCATG  
AGAGGTTTCAATAGATGGAGGGCTCCGCCGTTTTCCCGGCTCCGAGGATT  
CAGTGACGTACGACGCCAATTCCTCTGAAACGCCTCTCTAGGTTTCAAGT  
CAGCCCAGACCCACTGGCAGCCGCCCTCGCTGCAGAGACAGCCAGCTGG  
GTCTTGAGGTTCTACAGCGAAGCAAGGGTCTAGAAAAAGCAGACGTCT  
CTGGAAAGGGAGAAGCAGCCGATGGATTGGCATAACGGCGACAGGAGATT  
CTCGGACAGTGGCACCAGGAGAGGGGTGGACAGAGACTGGTGAACCGAG  
CGGGCCCAGGAATAAGTCCACACCCACACGTACCATCTCGTTGTTTATTT  
ATTTTTTCTTTTTTTCAGGGCCACTCTGGGGCATGTGGAGGCTCCCCAGCC

FIGURE 6, CONTD.

AGGAGTCGAATCGGAGCTGCAGCTACAAGCCTACCCACAGCCACAGCGA  
CACAGGATCTGAGCCATGTCTGCAGCCTACACCACAGCTCCCGGCAATAT  
TGGATCCTTAACCCACTGAGCAAGGCCAGGGACTGAACCCACGTGCTCAT  
GGATACTAGTTGGGTTTGTACCACTGAGTCACAGTGGGAACCTCTTTAA  
TTTTAATTTTTGAAGGTTTCAAACTCTTTAATTTTTAGTGAGGTATAGA  
TTATATTACGACCATTTCTTTCTGACTTCGGTGCACGGCTTTTCAACAA  
ATGGGTGCTGGACCTGCTGGGTGCCTTCTTCAAATGAACCACAAGCCCTC  
CCTCGCGCCGTATGCAAAATTTAACTCGAGGGGCTCATAGACATAAACGT  
AAACTCTAAAGCTATAAAATTTCCAGAAGAAAACGTAAGGAAAACCTTTG  
GGGTCTTGGGCAAAGATTTCTTACCCATGACAGCAAAATTACAATCTACA  
GAAGAACTGGTGGCCTTTATCGGCATTTAAACACCTGCCCTTTGAATGA  
TGCTGTGCGCAAAACCGAACATGCAGCAAAACGGATGCAACTAGCAGGTCT  
CACACTCAGTGACCCACGTGAGAAAGGAAAGACAGCCACGTGACATCC  
CTTAGATGCAGAATGTAAACACGGCCCCCGTGAACCGACCTCAAGAGAG  
AGACAGACCTACAGACGCAGCAAATTTGGGGTTGCCGAGGGGGATGCCGG  
Contig 114 (3000 bp)  
TGTGAGACCCCTTGGCGGGCCAGGACCCCCCAAGGTGACCGAAGGCCTCA  
GCGCCCCCAGCGCCCCCATCCCOCTCTTTCCCGACACAGGATTTTTTCC  
CACCAAGCTCTGTTCCCTTGGTCAAGCTCTCACTTGAGCAGCCTCAGGGT  
CTCCCGGTGCCTGTATCCACGACAGCGTGACCTTCTTGGTGTGTAACCC  
AGGACCCACAGCTGGCCAGCCACGCCTTCCAGAGCAGCCCCGCCCCATCC  
TCAGAGTCCAGAGGAAAGGCCCCCATTGACCCAGAAACAAAACGCAGA  
GACTCTGGGACGCCAGCAAGAACGTACTGACTCCACCTGCTTCAGGC  
ACGGAGGCAGGGGTGGGTATGAGCGACCCCGTGAAGGGCCTTCTTGTG  
CATCGAGGGGCTTCCAGGGGCTCTAGACGGGGATGAGTGTGGCAACATG  
TCGCCGCATTACAAAAGACCTGCAGTCTGCTGGGATGGGTCCCCCGGC  
TAGAAAAGCAAAGGATTCAGCCAGTCGAGTAGGAGGCGGCTCGGAGG  
CTGCAGAGGCGCGGGGGCGCTGACCACCACTCGGCAAGCCCCGTGTTGG  
AGGGGACGCCCCGCCCCGCTGCAGCCGCTGCGCCTCCGGATAAGCTCCTA  
AGAGGCCGCGTGCCCCATGCACGCGCGTGACACACTCGCTGCCCGAGGG  
TCCTTCAGCACAGACCTTGTGGGGACGGAGGACCTGGCAGGGGTGTGGCT  
CTGGGGAAGGGGTCTGTCCAGGAACCTGTTCTGGATTGGGGGTGGGC  
GTGGATATCCCGTCCCAACCTACAGAAGGGAGGGGCTTAAAAAGAGCCCC  
TTTGGTGTGAGGGGCCAGCAATCCTTTGGCTTTTTCTTGGCCACTTGA  
GCTTGACGTCTGGTCACTGACTGGGAGCCAGGGCCAGAGGGGGCAGCCG  
GGCTGAGGCAGGTTTCAAGCCAACCATCTCTCGGCCACACTCCCGAGGTG  
GGCAGCTACGGGGCCCCCAGAGACACAAGCCCCAGGGGTCTTCCCCCCC  
GCCCCCTGCCCCAGATCACCAGGAGACCCAAGCAGCTCTGCCTCCCCGTG  
CCTGAGAAATGCCCATCTGGGTACCAAATCACCCTCCAGAAGGTAGA  
GTGGGGGGCCAGGACAGGGGGACCCAGTTACAGAGCCCCAGGCAGGCT  
TCCCAGGGGCGAGGGGACTCCGTTTGGGGCACAGACGGAGGCAGAGCGGG  
CTGATGGATTCTCCCCCGGTTCAAGGATGCTGGCTGCCTGGCCTCCAGGA  
GCCGGCGGTGCCATCTGATCTGATTAAGGCCTGCAGTCCAGCTGGGCGG  
GCACAGCCTGGGGGCTCGGCGGGCAGGGAAGAAGCGCTGTGCCCCAGC  
CGGTCAAGCTCGCTTTCTCTTCAATTCCTCTCCATTAAAAGTGTGAAAC  
CATTTATTGATTTTTTAAATCAGGACGTGCTGTCCGTGACACAGCAAAGT  
GAACAAAATCAGAGCAAAGAGAGGCCAGGGCTGAAGCCCCAGAGGGCGGC  
GCCTCCAATCCGGGTTGTGCCCGGGGCTCCAAGCCCTTCTTCTCTGG  
GGTCTTGGGCGTAGTGGCCAGGGCAGAATGCACCTGCCGTATCTTGGGA  
GGCTTGGCCATCGCTGGCTTCTGTCTCATGACGCACCGTCTGTTCCATATC  
TACGGAACAGCTTCGCATTAACAGGCAGGGGAGGCGGTTGTTTCTCCTT  
TATCTGCCACCATCGGCGCTGGGGCCACGTGGAGCCAGCCGGCTGACT  
TCCCGCTCGCAGCAGGGGACTGATTGCAGGAACGAGGACATCCAGCCCC  
CGCCTCTCAATGCCCCGGGTGCTGAGAGCATTTGCCCCAAACGGCTTGGG  
TGGGACAAGGATGGAGCTGTGCGCCAGGGGCTGGCTGGGGCAGAAGGG  
GGCCTGCCCCGTGTCTGCCCGTGGCCTCCAGCACCTCGGCTGCCAGGCTG  
CTCTGGAGAGGTGCCCGGGGGCCAGGGGCCAGGGGACCCCTGTTCTGCCC  
CACGTCTCTGTCTGTGCTGAAAGTTCCACCAGACGCGTGTATACCCTG  
GGAGTCAGGAGGATGGGGGATAGTTGGGGCTTGACGTCTGTTTCTGAAAA  
AACACCGTTTTCCCTGAAATATATATGTATTAATTTTTCGTCAAGATAAA  
ACTGTGTATAGTTTTTTCGTGATGAGAAAACGCATCCATCTTCTTAGAAA  
GCCTGAAGAGGTACAGGAGCCTATAAAGGACAAGATGACAGATGCCTCTA  
ACGCACACCAAATGTGCGGTGGCCCCCAGGGGACCGCATAGACGGGGCGG  
CTCCAGATGGCCACCGTGTGCGAGGGACAGGTTTCAAGGTGGCAGAGTAT

FIGURE 6, CONTD.

TCCTGGGGGGGGGGGCTCAGCGGTTCCCATTTCCCCCTCCCTTCCTTCC  
TTCATTTCTTTCCTTCTTTCTTTCTTTTGTGGTTTTAGGGCCGCACCCG  
CGGCGTGTGAGGTTCCAGCCTAGGGGTCTAATCAGAGCTACAGCTGCC  
GGCCTCCACCACAGCTCAGGCAACGCCGGATCCTTAACCCACGGAGCGA  
GACCAGGGATGGAACCTGGGACCTCATGGATCTTAGTTGGGTTTGTTCCT  
GCTGAGCCACAACGGGAACCTCAGCCATTTCCCATTTCTTGCTCCAGTTCC  
AAGAATTTCAATTCTTATTCCTGTTCTTTAAGGCCAGAGGGCAGACCCAC  
GCGGATCCCAGAAGCAGGGCTCAAGGATGCTGCTGTTGACTGTGTCCGT  
GGGCGGGGGGAGTTGATAAGAACCCCAACACAGGGTGGTGGCCAGCAAC  
GGGGGAGGGAGGAGGGGGGCTGGTGGGGAAAAGTCCCCTGAACCCCATGG  
GCTGCCCCCTCCAGGCTGGGGCAGACCCCGAGCCCCATGGCCCGAGGAG  
AAACGGTCCCAGCCCCAGGCTGGGGCTCCCGCACCCCTGCCCTGACCCCGC  
Contig 115 (1895 bp)  
TCATGGAAGCCCTTATCACACCTCGGATCCAAAACCCACTGCGCGAGTC  
CAGGGATAGAACTCGCATCCCCACAGACCCCTATGTTGGGGTCTTAACCAG  
CTGAGCCACATGGAACCTGGGTAATCTATTTTAGATGTTCTTAGGGTTT  
TTGGCCTTGCTGTACGTGGGGACGCTGCTGGGCCAGGGATCAAACCCGC  
GCCACAGCTGTGACCCAAGCAGAGCAGTGACAGCACCCGGATCCTTAAGCA  
CGAGGCCAGCAGGGAGCCCTGTGTTTAGATTTTGGTGAGGATACTGCGT  
GGGATTCAGGATATTCACCTTGGGGCTGTGGAATTGCCCGTGCCTGTTT  
AAGCAAAGAGAAATCCCTTCACTCTGTGTAACCTGTGGGGAAATCCTTTAG  
TCTCTTGAACCATTTGCGTGTGTTAAGAGTGGTAACCTTGCCACCATAA  
ATGCCAGACAGCGCCTTCTGAGATCCGCTTTTGTGCAATATCTGG  
TTTGAATGCTTTGATCGCCCGCACCAGACAGGGTGGGCGGACGCCGCCG  
GGGACCCGACGTGACCATCGTGCTTCTGTATCCGCCCTTTCTCCGGCACG  
CGCCCCCTGGTTGCCTCTGGCTGCTTTTAGTGGAGGAAGTGAAGCCTCGC  
CACCCAGACCCCGAGACCCGAGGACCCACAATGCTTCAAACACCTGCCCT  
CTGACTTTTACAGGTCAAGTTCGCCAACGCCGAATTTGCACCGATTGGCT  
ACAGAGAGCACGGTGGCGCCAAGCCTCCACTTGGAGTTTATAAGGTCTC  
CCTCCAGCTCGCAATGAAAATGAGCTGTGATAAGGCAAAGACAAAATTAG  
TATGAAATCCAGATGCTTCATCTACAATACAATGACCGCGGATTGCGGT  
CTGAGCGACTGAAATCAAGGTGGGCTTCCGGAGGGAGGCTGTAGAGGAA  
AGGCATTCACGGAGGCTCAGGTCCGAGAGGCTTCCACACCCCTAAGAGGG  
CTGAGACGGCAAGTAGGGACCAAGCCCCGAGTCGGGAGAGCTGGGCAGG  
AAGGAAGTCTGAGGTCACCCCCACCTGGGGAGGAACTGCCTAGAGAAGCG  
GGGGCGGGAAGCAGGGGATGCCAGTCCCAAGACAGGGACAGGGCGGAAA  
GGGCTCTCTGCAGGCCCTCAATGCTGCCACAGTGTCTCGTAAGAGGGAG  
GCAGAGAGAATTGACACCGGGGAGACCAGGGACCACGGAGGTGGAGACC  
GGGCTGCCCCGCGCGTGCAGTTGCTCCCGAAGCCGGCCCCCTCCCCCAGAG  
CCTTTGGGAAGAGGCGCCAACCTGCAGTTCTGCTACTCGGGGACAGGGAC  
AGGGACAGCCCCCTGGAGCCGCTCTTAGGGGAGCATCCCCCAGAACCT  
TCCTTAACAGACCATCTGGAGAGAGATGGGTCTGGGCTGCAGCTCCTGGA  
ACTGTTTGGCCACCCGGCGAGCACCAGTGGGTGCCAGCCTGGGCTGCCC  
AGCCTCAGGGCCGGGAGGGCTGAGGGCACTGGGGCCCGGCTCTGGGACT  
CCCCTGCTCCTGCCGTGCAGGACAGCCACCTCCAGCATCTGCTTCTT  
GCCACCCACATCCCCAGGACCGTCAGCCAGGCATGCCCTGGCGTCGGC  
CACTCACACCACAGGCCAGGAACCCAAGGGGGGCAACACAGAAGGGCAGTT  
GCCATCTGCAGATGGAATGGACAAACTGGGGTCCGTGATGATGGCAGGCT  
CTGGGCGCCCCGGGTGGCAGGGGAGCCAGGACTGTGCGGCCATCACAGGA  
AGGGCATGACGGGGTGAAAGCAAGAGTGGAACCTCTGCCACCCGCTTG  
CGCACATACCGGCCACCCTGCAGCCCCACCCCATTTGTTTGCT

FIGURE 7

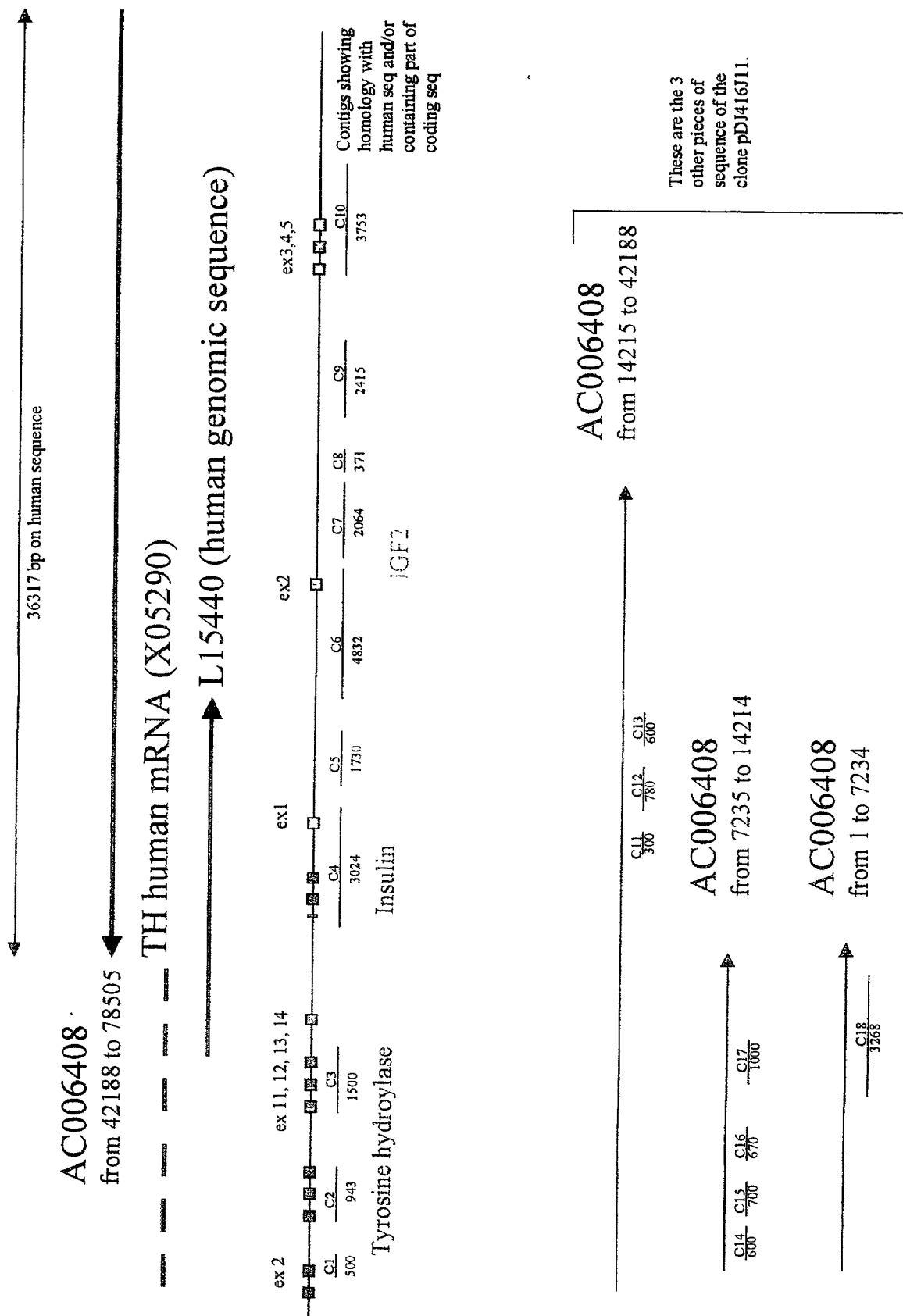


FIGURE 8

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Contig 1 (1040 bp)

GCGCGCCGGATCCTTAATTAAGTCTGAGAGATCTGCGGCCGCGGCCAGGGTCTGCTTCTG  
GCCAAGTGTGGGGCTCTGCTCCATCCTGGCTCGGAGGTCCACCCATGGCAAAGCCTGGGG  
TCCTCCCACTGAATATTTGGGGGTCCACTCGTGCCAAAGGCTGGGTGTCCAGTGTGCCAA  
CGGTACATGGAAGCAATGTCTTCCCAAGGACCGTCCAAGGTGTGGTCAGGCCTGGACAGC  
TGTGAGTCCCTTCGGGACTAGACTTGGTGGCCGAACCCTAGGGACCGTGCCCGAGGGCCC  
CCACGAGGCCAGGTGTTTGGCCCAAGGACAGAACGGCCAAGGGTGGCCGAGGGTCTTTTT  
TGTTTGTCTTTCTCTTTCTCTTTCTTTTGGCCGAGGGTCTTAAAGCGCTCTCTCTG  
CTCTTTGTCCCGATCCTGAGCGGGCAGTGTCTTGGTCTGGTGGGGTGTGGGCAGCCGAG  
CAGGGCTGAGAGAGCCCGGCTTGTCACTAGGGCGCGCCGGTGAAGCCAGCGGGCATGCCG  
TGTCCAGACGTTGGATGGGGCAGCGAGGGGACTGGGGTGCCCCAGCCCCCGTGGGAAGCC  
CGCCCTGTGGAAGCCGCTGTGCTGCCACAACAAGCACCGTCTGACTAGCTGGTGAATCAG  
CGCCCGTGCCTCCGTAATCCAGGCGCTTTCTGCCAACCTGAGCCCTGACCCACACC  
CCTTGCGACCGCTCCGTGGACCCCTGGGGCGATGAGGTGAACCGTGGGCTTGCCATCGTG  
GTGGCAGACGGTGGCACACCCGTGCGCCTGTGCGCCCCCTCCATCCAGGAGCAGAGTGC  
GCACCCAGTGGGGCTGGGCAGGAGCCGCTCCACCTCCGCCCTGAGGGGACGGGACTC  
TTTCGACCCGGAGTGGGAAGGGACATATGCGGACGATGCCAGACCCCTGTCTGTGGGGGA  
GGGGGAGAAGGCCCTCTTTGGAGAATTCCAGGACGGGTGAGGAACGTGTGCTGGACCGGC  
CGGGTCCGAGGTGGGCGCTT

## Contig 2 (9234 bp)

GGCAACCAGGGGAAGATGGGGAAGCGGGGTGCAGGGGCGTTTGGCGGGGCCAAGGACCAC  
CTTGGAATCTGGAGCCTGGCAGGAGCGGCGCAGGGTTGAGGGGCTGGCTTGGGCAGGGC  
TGGCTGGCACCTGGGAGCCTGGCGGGGTTGAGGTCCGGGCTCCAGGTGCCCTATAGGCA  
GGGCAACATCGGCATGGGGGTGACAGGCCCGAGCTGGGGTGCAGGAGGAAGAGGGGGGA  
GCCAGGCATTATCCCGGTCAATTTTGGTTTTCAGTCTGTGGCGGCTGGTGGTCAGGGGGA  
GTTGGAGAGAGGTTTCCCCCGGGGCTGGGGCAGCGGAGGTGTAGCTGGCAGCTGTGGGC  
AGGTGAGGACAGCCGTCTGCGGGGCCAGGTGAGTCCCTTCCCTCCCCAGGCCTTGTTC  
TCTGGCTCCTGCATCCGGAGGTTCTGGGGAGCGAGGGCCGGCGAGGCCAAGCGGCTGAC  
CCCCCGGCAGAGTGGCGGCGGACGACAGGCAAGGCGGGCAGAACAGGTGACACGTCTCAG  
GGGGAGCTGGGACCGGGCGGGCTGGGGGGCCGGGGCCGTCCAGGTGGAAGAGCATCT  
CAAGCGAGTCTGGTGGGAGACGAGGCAGGGCTGCCAGCAGGGAGGAGACGCAACAGGCGG  
GGGGCATTCAGGCCCCGGTGGGACAGGACCCGTGGGGGTGTGAGCAGTGGGGTCCC  
CAGCGCCACTTACCCACTGCAATTCATTAGTAGCAGGTACAGGAGCGGCTCTGGCCG  
GGCCTCTTGAGGCCTGAGCTGGAGCCTCGAGGGCCGGAGAATGGGAAAGAAGGTGCACTG  
TGCCAGACACAGCTACCTGGAGGGAGCACGGCCGTGGGGACGGGCCCCAGAGAGATTTC  
GGCAGCAGGAGGCTGCGCGGGCCCCAGCCTGCGGACGTGCGTTCCCACGCAGCACTGCGG  
CCCAGGGGCTGGCGCGGCAGGGCCCCCGGTGTCTTGGTGGCACTGTGCGCCCTCGCCGC  
TCGCCCCCTGGGACTGGCACGGCAGACAGGACAGCACCCAGGGGAGTCAAGGGCACTGAGC  
AGACCAGACTAGGCGAGGCGGGTGGGGTGGAAATGGATGTGACCTCTGGGGGGAGGGAGGT  
AGGGACCGAGCAGGGGCGAGGCGCGGAGCCTGGCGCGAGCGAGGCCAAGGCGGGCCT  
CTGCGGGTGACAACCTGAGCACATATGGGTACCTTTGCGCTCGCACCGGAGACAGGTGAGT  
GTCTGCCCCCGGCTGCCGCCCTCCCGGCCCCGCACTGCCTCTGCCCTCCCCCTCGACC  
AGGGCCCTCTGCTTCCCCACAGCCTCGTCTCCAGTGGGGGTGGACACACTGCCAGCACCA  
CAGGCCGGACGCCAGGATGTGCTTGGAGGGACATGACACAGTCCCGGTGTGACGGAGAGGG  
ACAGACGTGACGCCGTCCGGCCCTTCTGGTGAGCGCAGGTCCAGGCCTTGGCCCCCAGGC  
CAGCGCCCCCAGCCCCCAGCCCTCATGGCCGTCTTCTGTCCCGCAGAACACTCTCGGCTG  
GCCCCGCGGGGGAGCTGCCACACCCAGCGTCTGTTCCCTTTCCTTCTGAAGGAGCACGT  
GCATGACTGCTGCTCTTGGACCCAGAACCCCTCAAACGACAAGGTGAGGCAGGTCCCGC  
CTCGCCCCACAGTGAAGGGGCGTGGGCGAGAGCCGGGCGCTCACGGTGGCCCCCTCCC  
CCTGCAGAGATGGTGTACCCAGCTCATGCCCTGGGGCTTGGACCCGGAATCTTCAAGTC  
CTCCTAGCTCTGACTCAAGAATATGCTGCATTCTGGAGCCACTACACTACTTGACTCAGG

**SUBSTITUTE SHEET (RULE 26)**

FIGURE 8, CONTD.

AAGAGCAACGTCTGAGCTAGCTCCACGCGTGGGTCCATCTCGGCCAGGTTTAAATGAGCC  
 ACTTTCAGGCAGGGATTGCACAGGAGGCAGGGTGGGAAGTGGCTCTGCTCAGACCCCTGA  
 ACAGGGTCTGGAGATTCTCCAAGGGCACAAAAGAACGGACGATGCCCCGGGGTTCAGCGA  
 CAATGCTCCCTGAGAAATCTTGGCACACAGGGCTGGGCCTGCGAGGTGGCCCTCGCCCC  
 ACCCCAGCCTCCTGGAGGACAACCGTCGCCCTGCTCCAGAGCTGGGGGGCGCCACACGT  
 GGGGCACAGGAGCATGGGCCCGATTCCAGGCCCTGGGCTCCCTCTCGTGTCCAGGATCTC  
 CCCGTGTCTTGTCTCAACAAGCCCTGACTTGGAGGCCCCAGGGTGACCCCTTAAAGGGG  
 GAACAGAAGGTTCTAGAAGGAGCGTGGCCAGCTTTGGCTTCCCTAGGGCTGTGGTGACCA  
 CACTGGGCCACGGCCAGGCCACCCACCCGCTCCTTCCCCCTGGCCCCCTCCCTTCCC  
 CGACCTCTCCTGGCCTGCACCTGGTGACACGGCTGGCTCCCAGCCAGGGCTGAGGGGG  
 ACCAGCGGGGGCCCTTCTTGAAGCCACCTGCAGGCCGGCTTGTCTGGGAAGGGGCTGC  
 TCCTCGCCGGGCCACCCCGGCCGTTTCTTGAAGCGGTCACTGGATATTTTGT  
 CCTTGTGACGCGGAGCTTGCATAAAGCAGACACTGAGCTCCTTGTCTCCGGGAGCAG  
 CGCTCCATCACCGAACACCTGGCCGGACACAGGCGGGCAGCCGGGCTGGGGGAGCAGCG  
 CGGGCTGGGGCCGGACAGCAACGATCACGGCGCCGAGCGCAGGGCCCGCCGCTTC  
 TGCAGGCCGCCCCACGTGCCAGGCCAGCGGTGCCCATCCTGCAGGCTGGGAGGAGGC  
 TGTGGGCGCAGAGCTGAGAAGGGGGCAGAGGCACTGGGGGGGACAGCCGTGTTCCACA  
 CTTTGCAGAAACCTTGGCCGGCTGGATGTCTTGTGGGAGAGCTGGGGGAGGGGACAGG  
 GCAGGAAGCCGGTCCCCCGAGCGGGGTAGGAAGAGGCCTCGGCCCTGGGAGGAGGGA  
 GGGGAGGGCAGTGAGATGGAAGAGCACCAGGGGCTCGAGGCTTCTTCTGGAACAAGGA  
 CTAGAAGGAGGAGGCCGGGCGACTGCTTGGGATGCTTGAACAGGCCGCCCCAGTGCTG  
 ACAGGGACGTGACCTGGGGGCGGTCCCGGGCCAGGCGGGCTGGGAGGGCGCTGGTGG  
 GTGACGCCCACTCAGAGCCCTGGCAGCAGGGGGCTGGGCACGGCTGCAGGACAGAGCTC  
 AGGACACAGATGGGGCGAGGACTGAGTGGGGCACACAGATGCTCCAGGAGGTGGCCA  
 AGGAGTGGCCTTGGGATCCCAGGATGGCCCTGGTCCCAGAAGATGCCGCAGCCCAAGGGA  
 CCAGGCCAGGGCCGAGGGGGCCACAATCTGAGCAGGGCTCAGGCCAGGGCAGAGGCC  
 CCTCCCACCCAGCCCTCCCTGGGCCCGCTCTCC  
 GTGCAGGCACTGGGCTCAGATGGGGCAGACATGAGACCAGGTCCAGGGAGAAGCGGGGGC  
 CCTTGGCTTCATTAGGTGGCTTTTCCAGCCGCGCCCGTGGCTGGCAAGGCCACAGCGC  
 TCAGGAGCACACAGACCCCCACACGGGCTCCCCAGGTTGGGCGGTGACATCAGCCCTG  
 TGTCAACAGCAGGAGCTGGCAGCTCCCCACGGGGCTTAGGGAGCGGGACCCCTGAGCCA  
 CCCTGCCACCGCCCCACCCACCGTGGCCACACAGAGGCCCCGTGCTCTGGGTCTGGGG  
 CCAAGGCCCCCAGGCGCTGGCACTGTCTGCCCTCCCGCTGGCTCTCCGTCTCCAGTG  
 TCCCCGCCAGAGAGCATGGGGCCACAGGCCTGAATGCCACCTTCTTCTCCTCTGGAGG  
 GGGCTGAGGTTTTGGGGGTTACAGAGTGGCCTCCGGGGTGGGTCCAGGCCACGAGG  
 CAAAGCGGACCCAGGGAGTCCCGCGGAATGTGGGACAGCCCCCGTAGATCTCGGGGG  
 GGCCAAGCTCTGGTTGACCTCCATCCTGGGGCTGTGGGCCTTGGGTGAGTGGGGAGGGTC  
 ATGACACCCAGCCACAGCTGGTGACAGCCCTGGACGTGCCGGCTCAGGGCTGGCCTGC  
 CCTTGCAGCCTTGAACCCCTGTTCTCTGGAGTGGGGGCGAGGGGGCGCCGGGGCAGGG  
 TGAGAGACGAGAGCCTCTCTTCCCAGAACTTCTGCTGCGATGAGGACCCAGCAGGGCC  
 TCTCCTCACCAGAGGGCTCTGCCGGCTGCAGGGCCCCAGAGAGGCCAGAGGCTGGAGG  
 CCGGGCTTGGGAAGAGGCCGGAATCCAGAAACAGCTGCCCGCTCCGACAGACCCAGC  
 GCCCACTTGGGAGGGGGCGCGCCCCCGTGGCCCGCCGGGTCCACTGCTGGGGCCGCCA  
 CAATAAGTTTGTCCCTGCTGGTTACTGTCCGTGTCTGAGAGGTTTCTGGAGCCTGGCCA  
 CAATGGGCGTCAAGGATGCGGCTGGGAGGGAGCCTCGCGAGTCAAGTGTGCTGCTCGG  
 ACAGGCCCGCGCGCCCCAGCCCGTGTCTGTGGACAGATGGGTGGGTGGGTGGTGTCTG  
 GAGGGGGTTGGAGAGGTGGGCGGGACGAGGGGCTTCCCTGCACTCTGTCCCAGGGAAGCG  
 GGGACCAAGGAGGGGACAGCCCCCGGTACCAGGAGGGTCTGTCCCTCTCACCCCCCGG  
 GACAGGTGAGCTCCCCGAGCCGCGCTTCTGGGACAGGACCCACGGCCAGGCCACGGCC  
 CCCCCACCCCGTGGTCCCTCCGTCCACGGCCGGCTGGGGGGCCACGGGCCAGGGCC  
 CCCGCTCCCCGTGGCCCTCCGAGGGTGAACGACCTCGCCTGGGACGTGGGGCAGAGGGC  
 AGGCGCCAAGAGTGACCCCTGGGACAGTGGCTGTTTGCAGTTCTGGAGGCAGCCGAGA  
 TAAAGCGGCTGTTTCCCAGTGGGCTCAGGGCCAGAGGGGGGCGAGGGGCAGCCCCAGTC  
 AAGGCCGGGCGCTGCCTCGGGTCCCCCTCTGTGCGGAGGGAGGGGGCGGTTGCACAGC  
 AGCCCTGCCCCGCGCCCGCGCAGGCACCGTGGGACCCGGCCCTGGTGGCCCT  
 CCCCCGCCCCGCTCAGGGGGCCAGCCCTCTCTGGTTCCAGGACGCCCCCGCCCGCAGG  
 CGGCCAGAGAGTCCCAGAGTGTAGCCTCCACGTGTGGGATCCTGTATATGCGACAGC  
 TTAAGTCAAGGCCAATTTTCATGGGTCTGATTTGGGTGGGCACGGCCCTGCACAGCGG  
 GGCTGGAAGCCTAAGGCGGTGGGCGTGGGGGTGAGAGGCCCGCAGACAACAGGAGGGAGG  
 CTGGGACACTTCAAGGGTTGACATGCTATGCCTGTACGGATAAATGC

Contig 3 (5347 bp)

AGATGTGTATAAGACAGGGGCTGGGTGGGAAGGACAGAGGGTGGGGCCGGAGGAAATG

FIGURE 8, CONTD.

GGATGCAGAGCCCACCGTGCACGCTCTGCTGGCCTTTGAGCCTCGCTGAGTCGCAAGAAG  
CCCTCGGGCCTGGAAACAGACCCCCGGCCCCACCCCCACCCCGCCCCGATTACCCC  
GGCATGGCTGGAGGGCCCCGAGAAGCCACCCAGGCTTCCCGTCCCGAGCTGGGTGCTGGGC  
CCAGCCGAGCGGGCTTGACGCCACGCTTAGCCCTCCCCAGGGAGCCAGGGTCGGAAGGA  
AGAGGCCGGCCGGAGGGCCGTGGCCGCTCAGGCTGGAGGGGGCCCCGGGTGAGGATGGG  
CCCCAGACGTCCCCGCTCCCCGGCCATCCGTCACGGAGCTGTACCCAGGAACGTGCTCC  
AGACGTGCTTTCTGCGCGGAGCCCCGAGCAGGCTCCAGGCGCCCCACCCCCGAACG  
CCCACGCACACCTCGGTCTGCGAACACCTGCCGTGTCATCCGGTGGCCCCGGTTCCCGCC  
GCCCCGCGCATCCGGGTGCCCCCTTCCCTCCCTGGGTGGGGGGCCATGCCCTCAGCGGGCAC  
GCAGGCCTGTGAGGTCTGTTCTGACTCTTCCCCAAAGACGCAGGCGGCTGCGGGCGCC  
CCGACCTCGTCTGAGGCCCGTTTGTGCTCACTGGCTGTCTCAGAAAGGGGTGCCACGGG  
AAGCGCGTGTTCCTTGGGGCCGCAAGGCAAGGGAGCCCCACCCAAGGTGGCTGAGGGCAAA  
TGGCCCAGGGCCTTAAGGAGTCCCTGGGGGCCGGGGCCGGCTGCAGCTTGAGGAGGAGA  
GCCCTGGCTCTGCTCCCCGGGCAGGTGAGCCACGGCAGGGGGCTCCCCAGCAGCCTTG  
GCAGGAAGCACTGAGGAAGGGGTGAGGATGAAGCAAGGGGGCTGCGGGGACTTGGGCA  
AAGCCCCGAAGAACTGAGTTCTCGGAAAGGCCGGAGCCCTCAGCCGAGCCTCGGCCCTC  
CGAGCGATGGAGGCGGCCACCTGCGGGCCCCAGGGTGCAGCTGTGCATCCGTCCCCCTCG  
GGCCTCCCCCTGCCCCCGGCCACCACACTCTCCCCCTTTTGCTTTGATCACTTGAGT  
GCGACAGCTGTGCGGCCCTGAGCCCCAGAGACCGCTGCCCCCTGCCGCCAGCCCCACGG  
GAGCGTCCACCTGGGCCTGGCTGGGCACTCATCCCTCCCGATGAGGCCTTTCTAGCCT  
GGGCGCCCCGGGAGCGGCAGACCCAGCCCTCGCCCCCTCCCCAGTGAAGGTGCTGCT  
CTGGTGGTCTGGGGAAGCCCTGGAACAGGGGGCGCAGGTCCACACGGGTGCTCTGGCC  
TCCAGTGCACAGGAGGGCGCGCTCAGGCCAGGGTCCCCCACCAGAACCGCCAGGGC  
CCTGGGAAAACCTGTCTGTGCTAACAGGGCCGCTCCCCGGGACTCCACGGAGAGGTGCG  
AGGGACCCCTGAGCACCCACGCCACTAAGGGGGCCAGCCAGCTCGCGGGTGCAGGCAGC  
CGGCTGGGCGCTCACATGCTACTGCTCTCTGGCTTTGTGTGTGCGCCTGGGTTGGGGTG  
AGCGGAGGTGCCCGAAGGCGGAAGAGCCACCCCTCCACTCGGGGACCTATTTACGCAAGA  
AGACGGATGGGACTGCCGGGCATGGACAAAGGAACAGGATGAACCTTCTGGAACGCACAA  
GGCTTCCACGGCTGACCGGTGATAGGAAGGCGCTCTCTAGGCCAATCCACCGTCCACCG  
TCCATTTCCCCAGCCCTCGAGAGGGGGCAGGATGGACCGCTGCAGCGTGAGAGAGCTCTGG  
GGCGCTCCACAGGGCAAGTCCAGGGCACTGACCTCAGAGCCCAACCAGGCCACCGGG  
GCTGGGGCCACAGGGAGCCGGGGCCAGGGTCAAGGTGAGGGCCAGAGTGCAGGAAAGG  
GTGGCGTGTGTGCTTGGGGCGGCGGCGCAGACGGCCCTCGCACCCCCGACAGCCCT  
GGAGCTGAGTGAAGCCCGGGGTACCTTGGCTGGGGTGGGGTCTCTGCGACCGGCAC  
CCAGCTCAGGTGATCTTGTGTACCGCAGAGGGGCAGGGGTCTGAGCAGGGACAGGG  
TGGGCGCGCAGGAAGCCCCCTTCTCTCTGAGGCTGCCCCGGCCCTGGAGCCTCTCTGGG  
GCATGCCACCCCTCTCACAGACGCTCCAGGAGCCCCACTTTCTGCTGCGTGGTGAG  
GGTGTCTCTCACCCGATTCTTGGCCCTGCAGGTGAGTGCAGTCCCTGCTAAGCCTGGGG  
TTGGAGCAGGTGCAGGGCATCACACACAGCAGCAGAGGCTGTGGGGGCCCTGAGAGGC  
GCTCCAGGTACCCCTCTCAGGGGGTGAAGCCGGGGTGAAGCCGGGACCTCGCCTGCCCC  
CAAAGCCGGCGCCCTCTCCCGCCCCCGACAGGGCCAGAGAAGCAGGTGTGGGGCGG  
CACAAACCAAGTCAGCTTCCAGATCCTGCTGGGGCCGCTTGAAACTCGAAGCCCCAG  
GCTGGGAGGTCTAGACACCCCTGCCAGACCGACAGCCTGGGCCTGGCTCACAGCTGCCT  
GGGGGCCAGGGGTGCACCTGCCCTGTGGGTGGGGTCAAGGGCAGGGAACCCCTCGGGA  
AGGTCCCCCAGGGTCAAGGTGGGGCTAAGCTCCGGTGACCTCTGGGAAGTCTGGGGCTG  
GGTTTTGTTCCAGAGGAGAGAGGGCCAGTAGCCTCAGAGGGGCTGTGGCACGGTGGGAA  
GGCCCCAGGTGACCCAGAGCGTGCAGCAAGCCCCCTTGACTGCAAAGC  
GCAAAGGGCAGAGGTGGGGTGGGAGCCTCGACCCCCGAGCCAGGTACACAGGGGGAAG  
GGCAGGGATCCGCGAGGGGCCACCCGCCACCCAGGCAGCCACAAAGCCTTTGGGC  
CCGGAGCCCCAGATGGGGCCAGCCAGCTCTGGGAACAGTCTTCCAGAAATTTCCAGCT  
CTGGGTACCAACAGGGCTGCCCGCCCCAGAGCCCTCGGGCGGGAGACCTTCCCCAGG  
GGGATCTCCTAAGTGGCAAGGCCGTGTGGGAGGGGCTGGTGAGAGGCCACTCTGGCGGGA  
AGACCCCCAGCCACCTGGAGCCCTAGCCACTGCCTGCTGCGGCTCCCTAGGGATCCAGG  
GCCATCAGAGAAGCTCCAGCGACACTGTTTATTTTCAAATGACACTTTTAAAGAAAACA  
GCCTCACCCAAATGCTTGGCCCTGAGTCTGGAATGTGCAGACAGACAGCTGCCCCCTCCC  
AGAGCCTGCACGGCCCTCCGGGTGGGGGAGGAGCAGGGGGCACCCCTGGGACCGGGCCG  
AGGCTGTGAGGGCACGGAACGTGTCTGTGGCCCTGTCTCAATTCCCGGTGCCAGTGG  
CCCCAATTCCAGCAGACCCAGCAGGGCCCCAGCTTGTCTTGGCTGGCCGCTGGTCTCT  
GTCACCCAGGCTGGAGTTCTGGAAGATTCTGCTCCTGCTCCCGTGTGCACATACCACT  
CCCCGGGGCAGCCCTGCACCTTCTGTTCTGCTGGGCTCCCTGCTGATCCGTGAGGCCCT  
GCAGCCCGCTGATCTTCCAGGTCTCTCCGAGCCCCCGCTCCAGGAAGCCCTCCAGG  
AGAGCTCAGGAGGGTGGGCTCCCTGCGCGCAGCTGTGACACCCCTGGGGCCACCCCGCG  
GCTGCTAGGTTCCAGGTTCCCCACAAGCCCTCGGGCAGAGGCTGGGGCGCTGGGTCCCTC  
GGAGACAACTGGCTCCGAGGCTTGCCTTAGACGGGTTTCCGGGAGCCCGTCCCCAGCGG



FIGURE 8, CONTD.

CACCCACTGAGTTTTGAACACTTGGCGCCACCCCCACACCCAGGCGGTGGCCAGGAGGC  
 CTCCTGGGCAGCAGACAGTCCGTGAGGTGGCCCTGGGGTGGCTCCTGACCTGGGCGCTGG  
 CCCAGCCCTGGGCACAGCTTTCAGATCTTGCCCTGCCGCTTCTCCAGGCTGCCTCGGCC  
 CCTCCCGCTGGGGGTGCCAGCTTTCCTGGAGGATGCCACCCCTTGCCCATGGTCAGG  
 GAGGGGCTGAGAAACCCACCTCGTGCCTCTGCCCGGCTATGCCAGGGGAACAGGTTTC  
 CCTCCCGCAGGAGGGGACCGAGTCCCTGACAGCCCACTGCAGAGGGGAGGAGGTGCCTGG  
 CTCTGCCCCAGCCCCACCAACCCCGTGGCTTCTGTTTCGCAGCCCAAAAGCACTAAA  
 GGCCGCAGGTCTTGAACATCAAAGACCCGGAAGTCCATTGTATTGAATTGAGTGTAAA  
 TGAGCCTGAGCCCTGTGGCTTGCCTTCCACAATTACCGCTGCCCGGAAGGGCTCCGG  
 AACCGACACAGCCCCAGGGCCCTTGCCCATGTGGGGAGCCAGGCTGGCCTGAAGAAG  
 CCCCATAAGGTGGACCCCACTTTGAGCCCCACGAGAGTGGGCCAAGGACCAGGTACAGG  
 GCTGCCAGGCTCTGGGCTCCTCTGCCTGCCAGGTGGGCTCCCTCGGGGCCAGCCTGG  
 CCTGCAGGACCTTCCCACGTGAGTTCCTCCAGCCTGGTATGAGCGTAGTGGACGGCAGCC  
 ATGCCAGCACTCAGGGCCTGAGGGACAGAGCGGGAAGTCCAGCCCCGGGTCTTCGGC  
 CCCTAGGATCCTTCTAGGTGGGAAGCCCAAGGGAGCAGAGGGGTGAACGCAGCTGTGTG  
 GGGCCCCAGGCTGCCAGCAGACCCCTCTGCTCCACTCCTCGGCCGAGTGGGCGCCGAG  
 ATGCCGGGGCAGTGCCATTTCCAGGGGCCACCGAGGCTCCCAGAGGGAGTGAGGCACG  
 AGCTGGGAGGGAGGGCGGGGGGCTGGGGAGGCAGAGAGCGGAGGCCGGAGGCCGCTGAG  
 GAGGCCCGAGGGGGCCCTGGAGTCAATGACCCAGGGATTATCGTGCTGGGTCTTTGCAAA  
 GTTGGCTGAGCAAACGCCGCGAGCCCAAGGGTCAGGGAGACGGGACTGGCGGGGGCCCCGGG  
 CCCCCCTTTCCCTTTCTGAAAAAGCCTGTTTCCAGGTCAAATCCAGCTCATGATCCG  
 CCCCCCTTTGGGACTGATGTTCAAGGGCCAGTGGTCCCAGCACCTCTGTCCACCGCCCCC  
 CCCACGCTCCCGGGGCCGCCAACCCCTGTGGGCTGCGAGGTGCGGGCACCTCTCCCTTCG  
 AAGCAAAGCCCTGCCCTGCGTGGGCAGCGTGATTTCCTGCTTCTTGGGGCTGCACCTTG  
 ACTGGGGTGGGGGGGTGG

**Contig 4 (1592 bp)**

AGCCCCCTCAGCCCCCTCCGAGCAGCTGCTGGGCTCAGCGGGCTCGCCCCCGATGTGCGGC  
 CCTCCATAATCAATCATGGAGGGCCGGGCCCGGGGGGGGGCGGCGGACCTGTAGCCAGC  
 TCCAAGGGCAGGGACAGCTGCTGTTCGGAGGGTTCCAGGGGCCAGCCCCACAGACAG  
 CGGCCCTCGGCCCCCTTCCCGAGGGGCACCCACGGAGGGCCAGACCGGAGGGACTC  
 GGGGCCAGAGGCCAGGGCAAGAGTGAAGGCAGCGCCGCTGGGAGCGGCGGTGAGCGGG  
 TCCAGGCTTCAGTTCCAAGGAGCCCCATGCCCTGAGCCCGCACTGAGCCCTGTGCAGCC  
 TGTGGGTGCCGCCGAGGCCCCGCCACCCGCCCGCCCGCCAGCCTGGGGTCAAGGAGGGAG  
 GGGGTGGCCTGACGGATGGTAACAGCTGCTCCCCCACCTCGCCGGCGTGGACAGGGCTC  
 GCTTCTCCTGCCGAGCCCCCGGCTGCCCCATCCGTACGGCCCCACCCAGGACTGTGCGT  
 CCAGCCTCCCTCCCTCAATCCCCCGCATTTTCCGAATTCTCGGGCACTGCTGCTTC  
 CTCCTCAAATTCCTGGCCCCCTCGCCCCATCCCCGCCATGGGAAAGGGCCGCGATGCCA  
 GGACACTTGCTCGTCTCGGCCGGGCGGGGGGAGGAGCAGCTGGCTGGGCCCGGAGCTGT  
 GAGGTGCGGGGGTGCCAGGGAGAAGGGCCAGATTAGGGGGCGTCATGGGAAAGCTGGGA  
 GGAACGCTACCCAGAGCCCCCTGCGCGCAGCCTGTGCTGCTCCCTCTCCGCATTTCTG  
 GCCTCTGAGTGTCTCCCTGGAGGGAAGGGACCAGTGTGCTGCTGCGGCTCTGGCTCTGCC  
 AGGAATGTCCATCTGTCCGGGCGGGTTACCTGGCTCAGAGCGTGGGTACCACTCATCC  
 AGCCCTGACGCTGCTCTCGGAACAGTGGATGGGCCAGGCGCCCCCGTCACACCCCGCA  
 GCTGGGCTCCACAGACGGGCCCGGATGGCCACGAGGTGGGGGGCGGCCCCAGGGCGAG  
 GCTCCCTCCTGGAAGGGCTAGAGTGTGGGCTGCGCGGAGAGGGAGGCCGAGCGCCAGGC  
 CAGGTGCAGCCCGGGGAGGTGCTGGTGGGGGCTGTGACCCACGTGTGCAGCTCAAGGGT  
 CCAGGAGCCCCAGGGACAGAGCCTCAGGGACAGACCCCTCAGAGCCACAGCAGGAAGCCTG  
 GTGGCAGTAGCTGGCGGGGCGTGGGGTGTGCGGCCCTGCAGACAGAGGCAGAGGCAGGC  
 TCCCTGCTGATGACAGGGGCTTCTCTGTCCCTGCGGGGCGGAGGGGGCCCGACCATGG  
 ACCCGGGGCTCCTCTCGCACGATTCCAGGCCAGCCTGGTCTCAGGCAGTCCAAGGTTG  
 CACAATGGTCTCCATCGTCCAGAGTTGCAGAGCCAGCACTCTCCCACTGGACGGCGGGCC  
 GGGGTGGGCTGCACCGCCGCTCAGGGCTCAGGGCCGCGGCCAGCCNCCGAGGCC  
 TTGACCTGTCTCTTATACACATCTCAACCCTG

**Contig 5 (831 bp)**

TGAGATGTGTATAAGAGACAGGCCTTGACCCTGGGCTGGCTCAGCTGCGCGCCCTCCTC  
 CTTGCAGCTCCGCTCGACCCATCCATCAGCCATTTTCTACCCCTTCTGTAATAAAAA  
 ACCCGAAGCGGCGTGGCCCGGTGTCCGCTGGGGTGACTGCGGCTGCTGCTGGTGGCTC  
 CCACCTGGGCCCGGCCCCCTGAAACACACACCCGGCGATGGCTTGGCCGGGGCCCTGGT  
 GGAGGGGCGGGGGGCTCGCTGCTTGTCTGAAATTTTCGGTCCACATGCCCCGAC  
 TCCTCTCCCGGCCACCCCTGCAGGCCCGGCGGTGCCCGGCCACTTTCCCGAAGGACGG

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FIGURE 8, CONTD.

ACTCAGCATTTCCAGGGCACCTGCTGATGGTGCCAGACCCCGGGGGCCTTCCCGCCGG  
GCGCGGGCCCCACGTGCCCCCTCCAGTGGCCACAGCGGGCCTGGGCCAAGGCTGGGAGTTC  
TGACCGGGCCTGGGGGAGGAAGGCGGGGGAGAGGGGACACTCTCCTGGCGGGGACGAGGG  
TGGGGGAGCAGGTGGGGAGTTCACAGCCGGGGCAGCGGGACGCCGCTTGGCTGCCCT  
GGGTCTCAGCCGGGGACAGTGGCCACCAGGAGAGAGACGGCAGACAGTACAGCCCACCCG  
TTTATATCCTCTCAGGCGGTCTGTGCTTTATTGGGGTAAATATGCAGGACATAGAACT  
CTGCCACTGGACCCCTTGGCCGGGGGACACAGCAGCGGCATTGCATGCTTCTGGGTGCA  
GCGCAGCCAGCACCACCGGCCAGAGCACCCCATCTTCCCGATCAACCGGAC

## Contig 6 (4634 bp)

CTCTGGGCTAGCACCGTGGGGGCTTTGCCAGAGTGGAAGTGAAGTGGGTCCACCCCGGAG  
CCCAGAGGGCGGTGAATGGGAGGCAGAGCCCATCTGGGAATGGACCAGAAGAAAGGGAG  
CGGGGGTGGGGGAAGGGGCATCAGATCCTGGTCTTCTTGTGCGCTGCGGTCCCTCTGC  
CACCCTCCCCGAAGCTGATCTGGAGCACACGCGTCGTTAAAGCCGCCATCGAGGGCCCA  
CTTCTGACAGACGGAAGGGGGCAGAGTGCCTTCCCTACCGGCCTCGCCCTGGGAAGGCCC  
CTCCCTGCAGCCAGGAAGCCAGCAGAGGTGACAGAGCCAGGGGCCAGGGCCCCAGGG  
ACGGGCTCGCGGCCCCGAGCCGGGGTCCCTTGGCGTCCCCATCCTCTCGTCTGGAGCC  
CTCCTGGGTGACCACAGGAATGTGCAAGGCGGCAGCCGGGTGGCGGCCGGGAGGCGGGTG  
GGAGGCGGGGCGGGTGGCCTCTTACGGGCGGGCCTGAGAGATGGGCGCCCGTCCGGCCC  
TGGCGTCATCGTCTCCGCTCTCTACCCACTGAGCAAAGACACACGAAATGAAGCTCGAA  
CGAGCACAGCCAAAGAACGGCCGTTTCTGTCTTTCTTCTTAATCCCTTTGGCTTAGGGT  
TTCCCGGCTGGACAGCCTGCCCAAGGACATGGGCATCCGTCGGGGGACATTACAGGCA  
GTGACCAATCCCAGGCCACCCAGGCTGTGCCCTGCGTCGTGGGCCATTCCCAGCCGGCC  
AGAGATGGAGCAGCCACTCGGGTCCCCGAGTCTCGGTGAGACAGTCAAGGATGGACCTT  
GGATGGAGACCGGCGTGGCGCCATGTCCTGGGTGAAGGAGGCGTGCAGGCCGTGTGGG  
GGACATGGTTGCTGTCCCTCGGCCAAACCATGAAAGCAGCCCTCTCCCCCAACCCCCA  
GCACCAACCCGGAGACCACTCGGCCGGAGCCAGCAGCGGCCACCGTCACGTCTCGGT  
GTCCAGCTTGGGACAGGTGAGTTCCAGATGTCCAGGCTGGAGCTGGTCTTGAAGATCC  
TAGGGGTCCAGCCAGCAGAGGAGGCCAGGTGAGAGCCCCCTGTGGTCTAAGGATGCA  
ACCAGGGGCCGGCGGGGTGCCTGCCCTAGAGGGGGTAACTCGGCCCTGGGGACAGTC  
ACCCAGGAGGTCCCCAGAGCCAGCTCGGAGGGCCACAGGTGCCAGAGTCCCACCTGG  
GGAAGGCTGCCCTCCTGCCAGCCCCGAGCCGGGCCCTGGCGCCCGCGTCCAGCCGG  
ACCCCGGGGAGATATTACCCCCCTGCCCGCTGAATCAGGAGGCCCGAGCCCATGTTT  
CAGTCTTTTCTCCCATCCAGCCCCCAGGAGAAGAGGTGCTGAAGTGGGTCCCCTGG  
AGGCTCTGAGCCCCAGAAGTGCCTCTGAGCAGACGGGCACTCTCAGACCAGCTCAC  
GCTGGACAAGTCAGCTCCTGCCCTGATGGGCCCTTGGGAGAAGCAGACATGGTG  
AGGAAAAGGCCCTGTGCCCTTACCCCTAATTCGCCAGCCCCAAGTCCCAGTGGGTGCG  
AGCTTCAACCTAAGCAAATAATTCGTGCCCTCTAAACAAACGCGGGGAATCCACCTGC  
CCTTCCCCCGCCCCCCCCC  
ACCCCTGGCCTTGACCTCCAAAAGCACTTGAGGGGGCTTTCTCCAGACACCCTCCAACCC  
CGACCCCATGAAGAAGGGGTGATGGGGCTGTTACCCCAACAAGCAAGAGAACGAAGCCCA  
GAGAGGAGTTGGCGTGGACAGCAGGGGTGAGGCCCTTTGCCCGAGGGCAGGGCTGGTG  
CCACCTGGGTGAGCGGCAGGCCCTGGAAGAGCAGCGGAAATGAGCACACCTGGGTCTCT  
AGAAGGTTCTTCCAGACTCTGGGGGCTGAGTCAATTTCAACACTCCTGGGCCGGGCAGGG  
CTTCTTCTTGGCCCCGAGGGACAAGGTCCCCTTCGTCCGGGGGGTACGGCCCCCTGGACCC  
CTGTCCCCCGCACCCACCTCCGCTGGTGAGGGCCGCGGCCAGCTCTGGACACAGATC  
CCTCAGAGCCCCTTCTCCCTCCCTGCTCCCTCGTCTTCCCAAGATGCCCGGCCCTCCAGG  
TGGGGCAGCCAGGCGGCAGAATGTGGTCCAGGCCTCTCGGCCCCACCCACACCCCCCTGC  
TCTGCCCTGACAGCCTCAAGACGAGGCACGTGCTGCGTCTGCGTCTGTCTCTCTCA  
TGGCACAAAACGGTGCCCGCCTAGCTTCCCCCAGAGAAGGGAGATCGTGCTCCCCGGACG  
GACCCCTGCTGTGCTGTCTCCCGCCCGGCTTACGGGCTCTCCCCAAGGGTGGCCGCG  
AGGAGGCCCTCGCTCCGCGCACGGGGCTCCATCCTCCGAGCCCGACAGGCCTCCGCG  
TGGTGGTCCGACCTTTCCTCCCAAGGCCCGCCATCCTCCTCGCGCTCCCCCAAACCTG  
CCTCTTTCAGCGCCCTTGTCCCCACGGAAGACCTCCACCCGTGCCATTACACGCTC  
TCGCCCCACCTCCAGCCACCCCCCTTCCCCATCCTCCTGGAAGCTCCCACTTCCTTC  
CCGTCTCCACGCGCAGAGGGTCAGAGCTCAGGGGTCTGGGGCGTGGAGATGGCC  
TGCCCGGGGGTCTCGCTGACCGCCTCTACGGAAGCTGTGCCGGGGGGTGGGGGTGTCTC  
TGCCCGAACGGCTGGAGGACGAGCCACATCCAGGGCAGCGGAACCTGCGTCTGGTCT  
GAGACGGAGAGGCTGGGTGACGGTGGCTGAGGGGCTGCACACAGCTTGGCTGGGGTCC  
CCTAGGTGACAACACTGGCTGAACACTCATTGCTGCTCCCTTCCAGGGTGACCTGGGG  
TCCCCGTGTGGCCCTCAGGGCACAGGGGGCCCCACAGGCCTCACAGAACCCTAGTGGG  
ACTGCACCCAGGGCCACAGAAGTGGGGGGCACTGGGGGTCCAGAAACACCCCAAC

FIGURE 8, CONTD.

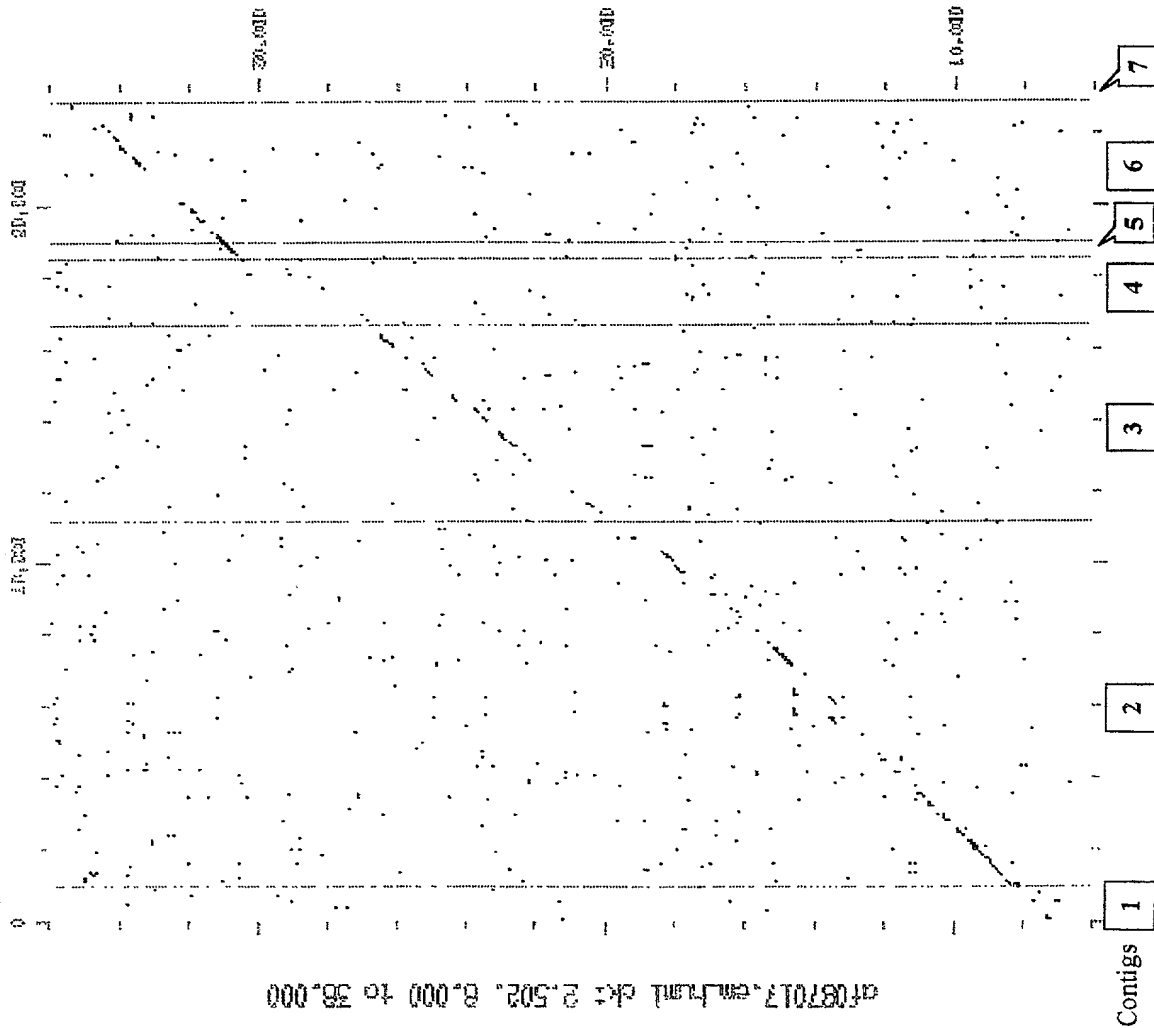
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GGATGGGGAGAAGAGGGCGCCCTGGCCATCTTGCTCCCCCTGGGACTTGAGGAGGGTCTC  
GGGCCGGGACAGCGGGACCGGGAGCCACAGAGACCCTGGAGGAGGCAGCATGGCGGGGAG  
GTGACCGGGGAAGAGGGCCGTGTCCAGGCTCACAGCCCGGCTGGCCGCCCGGCCCTCG  
GGAGGCGTGCCGCTGACCGCTGGCCGGGAGGTTTGCTGCGTGTGGGGTTTGCAAAAGT  
GCTGAGCTGCTGAGCCACAGGCCAGGCTCAGAGGGGACAGGAAGGAGGTTGCTGCCAG  
CCTCGGGCACTGCTGACCCATCTCCGTTTCCAGGGCACCAGAGCCACCTAATCTGCCGG  
CTCTGTGCCAGGGACAGGCTTGCTGATCTCTCAAGGCCGGGCGCTCCGCTTCCCTGG  
GAGAGGGTTAAACATCCAGCCCCAGCCAGCATCTCGGGCAGGTTCTTGCTCCCCCGCT  
CGTGCTCTCTGAGACCCTGGTCGGCACACCTTTCCTTGAGAGGAGGAGGAGGAGGAA  
AGCGGATGGAAACCAAGTGACCTGACGCCCTGAGGGCACCTTCCACGTGCCCCGCCCCG  
CCCCGCTCCTCCGCCCCAGTTCTCACGGCCCCAGTCTGATGGAGGGAGGGCGACCTC  
CGGGCTCCCTGGCTCCCGCGGCTCCGGAAGACAGGGCCGCTCGGCTGGGGCTGCAGGGA  
GGGGCCCGAGACGCAGGAGAGCAGCCCGGAGGCAAAACCCGCGGGTCTTCCAGAAGGAGG  
CCTGGCAGGGGGAGGGGGGTGCCACCACTGCTGTCCTCTCGTGCCACAGTGGAGGGTGT  
GGGTGGGCAGTGCCGGGTGGGAAGTGCAAGAACCCCTGGACCGTGGGGCTGGGGCCGCC  
ACGGGGGAGCGGGGTCTGTAGGGACCCCTGGGGGAGGGAGGCGAAGGGCTGGGGCAGAGG  
CCGGATCACTTCCAGATTTGCTGTGGGACCAAGGGCCGGACCTCGGGGTGACTTCTTTTG  
TGTGCTGGCCACAGGGGGGCCCCGGCGAGGTCACACGGAAGGGGGCTTCGGACCTGGCCT  
AACAAGCCCACTCCCGAGGAAGATGCAAGGGGAGGCAGACGGAAGGGCCGAAGGGGGCGA  
TCGGGGGACACCGCGCAGGGCCGGGGCAGAGAAGGGAGGCAGAGGGCAGAGAAGGGAGG  
CAGAGGGCAGAGAAGGGAGGCAGAGGGGCCACATGCTTGGAGGGCCAGGGAGGAGCGGGA  
ACGGCGTCCGGCGTCCAGCGCCGAATCAGGCCCGTCAAGCGGAGGGTGCCTGGACCTGCC  
TGGCCTTCACGAGCACAGTCAGCAGGCTGTCTTTATACACATCTCAACCATCAT

## Contig 7 (482 bp)

AGCAATGGGGCCGTGACCTAAGGAGGCAGGCCAGGTCAGTGGGGTGACCTCTCGTGGCC  
CCGATGTTTGGAAATCCCCAAATCAAAATGACCCATCCGACAAGCTTGATGCCTGCAGG  
TCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGCCCTATAGTGAGTCGTATTAC  
AATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCCAACTT  
AATCGCCTTGACAGCATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCCGACC  
GATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTT  
CTCCTTACGCATCTGTGCGGTATTTACACCGCATATGGTGCACTCTCAGTACAATCTGC  
TCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACCGGAACCCC  
TT

FIGURE 9

Human clone af087017.em\_hum1: H19 gene + flanking sequences



Human clone af087017.em\_hum1: H19 gene + flanking sequences

FIGURE 10

IDENTIFIED POLYMORPHISMS:POLYMORPHISMS TYROSINE HYDROXYLASE GENE - CONTIG C3 (figure 6)

1	GGATCCAGCC (A:T) GCAGCC	1081 bp
2	ACAACCCCC (-:C) TCCCACAG	1149 bp
3	TGCGGAGGGG (A:G) GACCTG	1186 bp
4	AGGT (CAAGGCCAGGT: -) CGAGG	1210 bp

POLYMORPHISMS INSULIN-IGF2 - CONTIG C4 (figure 6)

5	CCC (C:A) CCCC (A:C) CGCCGC	438 bp
6	CCC (C:A) CCCC (A:C) CGCCGC	443 bp
7	CGCCGCAGCA (G:A) GCCG	455 bp
8	GCTTATGG (G:A) GCCGGG	503 bp
9	CACGGC (T:C) TC (G:A) GAGCA	525 bp
10	CACGGC (T:C) TC (G:A) GAGCA	528 bp
11	GTCTGC (A:G) GGCAGGTG	571 bp
12	CAAGCCCGG (G:T) CGGTT	636 bp
13	ACCTC (A:G) AGGCCCCCA	710 bp
14	GC (C:T) GGGCCCAGCCGC	867 bp
15	ACCAGCTG (C:T) GTTCCC	903 bp
16	GGC (C:G) CTCTGGGCGCC	1148 bp
17	GGGGG (C:T) GTCCCGGGA	1305 bp

## FIGURE 10, CONTD.

18	GCGGT (C:T) GGGGGAGTT	1320 bp
19	CGCCC (C:T) GGTCCCGCT	1400 bp
20	TCCC (G:A) TCTGCCGGCC	1519 bp
21	GA (T:A) GCCCCATCCCCC	1547 bp
22	GG (C:T) GGCTGCTGCGGC	1607 bp
23	TGGCTGC (G:A) GTCTGGG	2222 bp

POLYMORPHISMES IN CODING REGION - CONTIG C10 (figure 6)

24	GCGCA (G:T) TGATTGGCA	341 bp
25	CGCCCCCCCCC (-:C) (G:C) GG	2247 bp
26	CGCCCCCCCCC (-:C) (G:C) GG	2248 bp
27	GCAGCCGGCTC (C:T) TGG	2257 bp
28	GTTGTTG (C:T) TCTGGGA	2413 bp

MICROSATELLITES

29	PIGQTL1: (AT) <sup>11</sup>	112 to 133 bp Contig 57
30	PIGQTL2: (GT) <sup>8</sup> GCACGCGTGCGTGTGTAC (GT) <sup>17</sup>	1074 to 1144 bp Contig 95
31	PIGQTL3: (CA) <sup>19</sup>	223 to 260 bp Contig 105

**Declaration and Power of Attorney Patent Application  
(Design or Utility)**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: 'Selecting animals for parentally imprinted traits'

the specification of which

- ☐ is attached hereto  
x was filed on June 15, 2001 as application serial no. 09/868,732  
and or PCT International Application number PCT/EP99/10209 and was amended  
on (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information know to me to be material to patentability as defined in 37 C.F.R.§1.56.

I hereby claim foreign priority benefits under 35 U.S.C.§119(a)-(d) or 35 U.S.C.§365(b) of any foreign application(s) for patent or inventor's certificate, or 35 U.S.C.§365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate of PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)		
Number 98204291.3	Country EP	Day/Month/Year Filed 16-12-1998
Number	Country	Day/Month/Year Filed
Number	Country	Day/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

Prior Provisional Application(s)	
Serial Number	Day/Month/Year Filing Date
Serial Number	Day/Month/Year Filing Date
Serial Number	Day/Month/Year Filing Date

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s), or under 35 U.S.C. §365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

Prior U.S. or International Application(s)		
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



## Power of Attorney

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Joseph A. Walkowski	<u>28,765</u>
Kent S. Burningham	<u>30,453</u>
Brick G. Power	<u>38,581</u>
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Thomas J. Rossa	<u>26,799</u>
James R. Duzan	<u>28,393</u>
Robert G. Winkle	<u>37,474</u>
Kenneth C. Booth	<u>42,342</u>

(14)

I hereby authorize them or others whom they may appoint to act and rely on instructions from and communicate directly with the person/organization who/which first sends this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instructed otherwise.

Please direct all correspondence in this case to at the address indicated below:

TRASK, BRITT, & ROSSA

P.O. Box 2550

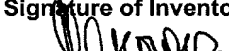
Salt Lake City, Utah 84110

Full Name of Sole or First Inventor		
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Residence and Citizenship		
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Post Office Address		
Street Address Bergagatan 30	City Uppsala	State & Zip Code or Country S-752 39
Signature of Inventor <i>[Signature]</i>		Date July, 21, 2001

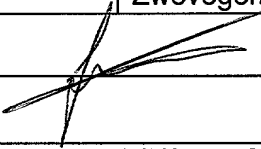
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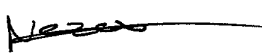
2-00

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Residence and Citizenship		
City of Residence <u>Villers-aux-Tours</u>	State or Country of Residence <u>Belgium</u>	Country of Citizenship <u>Belgium</u> <u>BEX</u>
Post Office Address		
Street Address <u>Rue Vieux Tige 24</u>	City <u>Villers-aux-Tours</u>	State & Zip Code or Country <u>B-3161</u>
Signature of Inventor 		Date <u>13-8-2001</u>

3-00

Full Name of Third Inventor, if any		
Family Name <u>Spincemaille</u>	First Given Name <u>Geert</u>	Second Given Name
Residence and Citizenship		
City of Residence <u>Zwevegem</u>	State or Country of Residence <u>Belgium</u>	Country of Citizenship <u>Belgium</u> <u>BEX</u>
Post Office Address		
Street Address <u>Sint Denijsstraat 26</u>	City <u>Zwevegem</u>	State & Zip Code or Country <u>B-8550</u>
Signature of Inventor 		Date <u>13-8-2001</u>

4-00

Full Name of Fourth Inventor, if any		
Family Name <u>Nezer</u>	First Given Name <u>Carina</u>	Second Given Name <u>Danielle. A.</u>
Residence and Citizenship		
City of Residence <u>Neupre</u>	State or Country of Residence <u>Belgium</u>	Country of Citizenship <u>Belgium</u> <u>BEX</u>
Post Office Address		
Street Address <u>7, Impasse des Bruyères</u>	City <u>Neupre</u>	State & Zip Code or Country <u>4120</u>
Signature of Inventor 		Date <u>13-8-2001</u>

# SEQUENCE LISTING

<110> Andersson, Leif  
Georges, Michel  
Spincemaille, Geert  
Nezer, Carine

<120> SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS

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<140> US 09/868,732

<141> 2001-06-15

<150> PCT/EP99/10209

<151> 1999-12-16

<150> EP98204291.3

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<210> 23  
<211> 450  
<212> DNA  
<213> Pig

<400> 23  
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ggggtgggga gcgcaaggca tggcctgtgt gcccagccc agtccttcag ggcgctgtg 180  
tctgcaccg ggggccccag gaagcagagc acccagcttc tcccctattc tagaaccagc 240  
ccccagaacc ctggaccag acccaggccc aggggatact gacagagcca cggaaggcg 300  
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ggggcagatg ggacacgaga gcagccctca tccacagcag gcaggggagt gaactggtgc 420  
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<210> 24  
 <211> 535  
 <212> DNA  
 <213> Pig

<400> 24  
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 gcctccacgc ccaacatcag gcctgctgca gccctggacg gccccctgtc ccccggaagc 180  
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 catatgcaca ggatatggat tgggtgtgga tttaaccttt tcgcaaagt ctctgccggt 360  
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 ggagctgggt tgctgaacct gccctggccc ccacccccaa gggagccccc ggccagtgtct 480  
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<210> 25  
 <211> 868  
 <212> DNA  
 <213> Pig

<220>  
 <221> misc\_feature  
 <222> (310)..(310)  
 <223> Nonspecific nucleotide

<220>  
 <221> misc\_feature  
 <222> (857)..(858)  
 <223> Nonspecific nucleotide

<400> 25  
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ggccaggggtg ggcagcccca gcctcagcag ggcgtctctt ggggctcagg ctgcgccgac	420
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cgcgctcctt catgggggtgc taatcacgga gtaaggagcc agagaatgag gcacggagta	660
tccagtgtta accctggagt atggagacgg gagtactaat tgtggagcat ggctctaagg	720
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caacggggac ggggtatccg aaggggagga tggagtatcg gccggagggg ggagaatgga	840
cactagagga tgtatanngg gcgtcaat	868

<210> 26  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 26	
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ttgattgaac ataacggtaa atttggcatc gttatcccgg gcgttgacgc aactaactgg	180
acgtgggcgg tgggaagtgt gtcggggcgt gatgaagata aatttaattg ctatggcatt	240
ccggttgtga gaggcccggt atttggtttg cctctgggtc aggaaaaatg tctggcgtgg	300
atggagtgtc gattgctacc tgcgacttct gcgcaagaag aatacgacac gctgtttggc	360
gaagtagtat cagcagcggc agacgcacgg gtatttgtcg aaggccgctg gcagtttgat	420
gatgataagc tcaatacgtt gcatcattta ggtgctggga cgtttgttac cagcggcaag	480
cgtgttacgg cgggttaagc	500

<210> 27

<211> 900  
<212> DNA  
<213> Pig

<400> 27

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tcgtaatctt cagtaagact ctgcacgtcg aaagcaatac cgtcaccgtc agctaacagt 180  
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aacttgcggt taatgcggcg taccagat acgacatcta taaaaaaatc gtccagcccg 840  
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<210> 28  
<211> 500  
<212> DNA  
<213> Pig

<400> 28

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gtccctgggc acgggcccgc tcctgacgca ccatgtgttc aaggcaagag cactggacag 180  
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cctgctctc gccgcgtgc acggacctct cccggcccc caggctcatt actcttaagg 360

accctagccc cctatgctga aatgctgtac ctcgtgcttg ttttcatctg tttattacct	420
tatottcatt cctgcttgat gatatactggg tattctttat tgattatata tatcttggtc	480
gtgtttttat aggacactgt	500

<210> 29  
 <211> 450  
 <212> DNA  
 <213> Pig

<400> 29	
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gcggtcatac cgaactcgcg aacaccgtag tggatgtagt taccgcgagc atcttcggtg	360
attgcttttag aaccagacca cagggtcagg ttagacggcg ccgggtcagc agaaccgcgcg	420
aggaattccg gcaacagccg gacgaacgct	450

<210> 30  
 <211> 450  
 <212> DNA  
 <213> Pig

<400> 30	
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ggtcggtacc gccagaccg agggcctcgg ggcccgggtg accccagctg tcgcacacgc	360
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ccccgcctgg accccatgaa gccccgcgga	450

<210> 31  
<211> 600  
<212> DNA  
<213> Pig

<400> 31  
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<210> 32  
<211> 450  
<212> DNA  
<213> Pig

<400> 32  
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aggacggcgt gtatgaccgt ttgcccga aaattgcagc ggcaatgagc aaactgcaca 360  
tcggcgacgg gctggataac ggcgtcacca tcgggccgct gatcgatgaa aaatcggtat 420  
caaaagtgga agagcatatt gccgatgcgc 450

<210> 33  
<211> 450

<212> DNA

<213> Pig

<400> 33

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cgcccgcgaa atcaagatac tcacggcctt 450

<210> 34

<211> 500

<212> DNA

<213> Pig

<400> 34

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tcagaagcag accccaggca tcgtaatatg tcatccgtga gttccctgtg tgccaccaac 480  
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<210> 35

<211> 400

<212> DNA

<213> Pig

<400> 35

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aggacctcta ctgttctgc ttatcctccc ctttgagcca	400

<210> 36  
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 <213> Pig

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 <211> 500  
 <212> DNA  
 <213> Pig

<400> 37	
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 aatgaggtcc agttcatcca gtttacgacg ggagaggaca ggggagattt gttcgatgac 420  
 cggaagggca aaaattttct taatcatgac gcagtccttt aacttcattt tatcaggtaa 480  
 aaaaaagagc gaccgaagtc 500

<210> 38  
 <211> 300  
 <212> DNA  
 <213> Pig

<400> 38  
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 agccggttca gggtagcct gataatttgc attttaaata ccatttattg ggtacttttt 300

<210> 39  
 <211> 450  
 <212> DNA  
 <213> Pig

<400> 39  
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 gccactgtc gtctaatacat ggagaaattg tccatcagtg gggctctgat gggcagggga 120  
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 aaacctgatt ttctcctttc taagccgcta cagatttggg agcatattca cctttaatcg 420  
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<210> 40  
 <211> 450

<212> DNA  
<213> Pig

<400> 40  
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gccagcaggc aacaaatttt tatgattttg 450

<210> 41  
<211> 400  
<212> DNA  
<213> Pig

<400> 41  
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ctgaacgcaa tcgttacgag tgtaaatagt aatgcgcgat attcgtatct ccgtttaaaa 120  
tgaagatacg gcgcgatgat acgcgtcggg ttgtctctct gttgatacag agatactaga 180  
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<210> 42  
<211> 500  
<212> DNA  
<213> Pig

<400> 42  
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<211> 400  
<212> DNA  
<213> Pig

<400> 43  
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<210> 44  
<211> 450  
<212> DNA  
<213> Pig

<400> 44  
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<210> 45  
<211> 750  
<212> DNA  
<213> Pig

<400> 45  
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<220>  
<221> misc\_feature  
<222> (299)..(299)  
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<210> 47  
 <211> 300  
 <212> DNA  
 <213> Pig

<400> 47  
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 tccctactgt gtaggcacca gggcagagtc agccctggga ccatggccac agctgctccc 180  
 gcctgagccg ggccccccgc ccaggctggg cccctcagt gcactgtccc aagccagctg 240  
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<210> 48  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 48  
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 <211> 500  
 <212> DNA

<213> Pig

<220>

<221> misc\_feature

<222> (500)..(500)

<223> Nonspecific nucleotide

<400> 49

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<211> 600

<212> DNA

<213> Pig

<400> 50

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<212> DNA  
<213> Pig

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<212> DNA  
<213> Pig

<400> 52  
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<213> Pig

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<210> 54  
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 <212> DNA  
 <213> Pig

<400> 54	
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<210> 55  
 <211> 1133  
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<400> 55



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 <212> DNA  
 <213> Pig

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 <212> DNA  
 <213> Pig

<220>  
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 <223> Microsatellite PIGQTL1: (AT)11

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<212> DNA  
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<400> 61  
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<400> 63  
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<210> 64  
<211> 450

<212> DNA

<213> Pig

<400> 64

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<213> Pig

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<212> DNA

<213> Pig

<400> 66

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 <212> DNA  
 <213> Pig

<400> 68  
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 cctcctccag gaagccttcc agggctgcct cggcctgggtc tcagcaggag acagagagag 480  
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 ccctggggccc 550

<210> 69  
 <211> 500  
 <212> DNA  
 <213> Pig

<400> 69  
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<210> 70  
 <211> 550  
 <212> DNA  
 <213> Pig



<400> 70  
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 <211> 1300  
 <212> DNA  
 <213> Pig

<400> 71  
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<210> 72  
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 <212> DNA  
 <213> Pig

<400> 72	
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<210> 73  
 <211> 550  
 <212> DNA  
 <213> Pig

<400> 73	
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<210> 74  
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 <212> DNA  
 <213> Pig

<400> 74  
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<210> 75  
<211> 450  
<212> DNA  
<213> Pig

<400> 75  
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<212> DNA  
<213> Pig

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<223> Nonspecific nucleotide

<400> 76  
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 <212> DNA  
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<400> 77	
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cgccaccggg gggggggggg gggggg 626

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<211> 500  
<212> DNA  
<213> Pig

<400> 79  
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 <211> 427  
 <212> DNA  
 <213> Pig

<400> 80  
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<210> 81  
 <211> 650  
 <212> DNA  
 <213> Pig

<400> 81  
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<211> 550  
<212> DNA  
<213> Pig

<400> 82  
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<210> 83  
<211> 550  
<212> DNA  
<213> Pig

<400> 83  
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<211> 984  
<212> DNA  
<213> Pig

<220>  
<221> misc\_feature  
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<400> 84  
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<210> 85  
<211> 550  
<212> DNA  
<213> Pig

<400> 85  
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<212> DNA

<213> Pig

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<211> 1040

<212> DNA

<213> Pig

<400> 117

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<211> 9236

<212> DNA

<213> Pig

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